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FACTORS IN THE PATHOGENESIS OF PYELONEPHRITIS

In 1916 Cabot and Crabtree made the following statement about bacterial infections of the kidney:

> While it is almost literally true that a satisfactory comprehension of the diseases of the genitourinary tract depends upon a clear appreciation of the nature and probable course of renal infection, there is no subject in which there is so little uniformity of opinion and so much confusion. The literature of the subject is stupefying both in quantity and in complexity, and anyone who has attempted to master it will, we think, be convinced of the fact that it is more likely to confound than to enlighten the reader.[2]

In the succeeding forty years some progress has been made, the most important being an appreciation of the long, indolent course which may be taken by this kind of disease. The misleading word "pyelitis" has been replaced by the more accurate "pyelonephritis," and the tendency of the disease to cause serious destruction of the kidney is now well recognized. Furthermore, good evidence has been provided to implicate pyelonephritis in the etiology of some forms of hypertension and some cases of toxemia of pregnancy.

However, there is still much uncertainty and confusion about the pathogenesis of pyelonephritis. Problems which should be susceptible to study by accurate clinical observation or laboratory experiment remain unsettled. An example is the ancient mystery as to whether bacteria reach the kidney by way of the blood stream, the lymphatics, or the urinary passages. Adequate information is lacking on the microbial population at various levels of the normal urinary tract. Although pyelonephritis is known to occur with special frequency in certain age periods and to be a common accompaniment of certain diseases and conditions, the causes of these associations are difficult to define. It is not known whether the pyelonephritis of an infant should be regarded as the same disease as that which may be encountered in a patient with spinal cord transection. We do not know whether the course of pyelonephritis varies depending on the kind of causative micro-organism, or why infection occurs so much more commonly when there is obstruction to urine flow. The risk of upper urinary tract infection following cystitis cannot be stated, and, conversely, it is not known how often cystitis occurs

* Ensign Professor of Medicine; Chairman, Department of Medicine.

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as a sequel to kidney infection. It would be important to know how often such procedures as catheterization and cystoscopy lead to upper urinary tract infection, and also why treatment with potent antimicrobial agents only occasionally results in eradication of the infection.

In preparing this article an attempt has been made to bring together and appraise many of the clinical observations and laboratory experiments which bear on questions such as those listed. Here and there the collected evidence seems adequate to permit the forming of a tentative conclusion, but more often the answer is still elusive, partly because no information is available and partly because the "data" are inadequate or undependable. Attention will be called to certain peculiar features of this infectious process. It is hoped that the material as presented here will not also be "stupefying" but will instead help to define some of the problems and provide stimulus for further productive studies of an immensely important disease.

SOME NOTEWORTHY FEATURES OF PYELONEPHRITIS AS AN INFECTIOUS PROCESS

The micro-organisms commonly associated with pyelonephritis (E. coli, staphylococcus, enterococcus, etc.) are pathogens capable of producing acute pyogenic infections in many parts of the body. They are usually looked upon as extracellular parasites susceptible to phagocytosis and lacking any notable tendency to lurk in the tissues for long periods of time. Although at times pyelonephritis exhibits the characteristics of an acute pyogenic infection, its outstanding characteristics are indolence and extreme chronicity, comparable to the usual behavior of pulmonary tuberculosis. It is not uncommon for this infection to proceed to a stage of severe renal damage without any preceding illness or disability which can be identified in retrospect as having been a manifestation of acute pyelonephritis. The histological picture found in an established case is not typical of an acute pyogenic process or of a viral or granulomatous disease; it is compatible, instead, with the indolent clinical course of the disease: some cellular infiltrate, some scarring, but bacteria rarely demonstrable.

Considering the fact that the responsible micro-organisms are usually susceptible *in vivo* to the action of sulfonamides, nitrofurans, or antibiotics, the results of drug therapy in this disease are surprisingly poor. Only about 10 per cent of cases of chronic pyelonephritis respond satisfactorily.[35] Consider, for example, the results in enterococcal infections of the urinary tract. The enterococcus is susceptible to the combined effect of penicillin and streptomycin, these drugs appearing to have a bactericidal action *in vivo* as well as *in vitro*.[36] Nevertheless, enterococcal infections of the urinary tract are eradicated only occasionally by this same supposedly bactericidal com-

bination of antibiotics. Yet the factors responsible for the poor response to chemotherapy are not obvious. Inadequate blood supply does not seem likely since even the "scarred" kidney is not the avascular structure we find in a vegetation of bacterial endocarditis or the wall of a chronic draining sinus. As a surface on which phagocytosis can take place, the kidney does not seem to share the defects noted on serous surfaces or in fluid media.[88] The indolence, chronicity, and resistance to chemotherapy bring up the question whether the causative bacteria may at times lodge in some kind of shelter in the kidney, perhaps establishing a form of intracellular parasitism there. The very indolence of the infection may be partly responsible for the indifferent results of chemotherapy, since these agents are often most effective when the micro-organisms are in a stage of active multiplication.

Undoubtedly one of the important factors in the problem of kidney infections is the matter of frequent or almost continuous seeding of the kidney with fresh supplies of bacteria. All clinicians have experienced the frustration of watching an infection due to one kind of microbe subside, only to be superseded by infection due to another. Even with persisting infection due to, say E. coli, there remains the possibility that fresh strains not differentiable by common bacteriological methods are entering the picture from time to time. It is impossible to determine whether these changing bacterial populations represent *new* infections, or whether they are simply alterations in equilibrium whereby the suppression of one form permits growth of another.

The interrelation between bacteria growing in the urine and bacterial infection in the tissues of the kidney, ureters, and bladder is not understood. The question is often raised whether the aim in chemotherapy is eradication of the bacteria in the tissues or those in the urine. Prevailing opinion seems to emphasize the importance of directing treatment toward bacteria in the tissues, but this may not be correct, since it may be necessary to prevent growth in both areas. Undoubtedly phagocytosis of micro-organisms in urine is not very effective; this may be one of the factors which makes sterilization of the entire system so difficult.

Of central importance is the rôle of obstructive uropathy in contributing to the occurrence and stubborn persistence of kidney infection. This problem will be considered in more detail later, though without satisfying answer.

BACTERIOLOGY OF THE NORMAL URINARY TRACT

The urethra. The meatus, being on the surface of the body, and contiguous with skin heavily populated with bacteria, naturally always has bacteria on its surface. We need more information, however, on the bacterial flora of

the urethral canal. Textbooks of bacteriology usually state that it is normally sterile or may contain a few micrococci, diphtheroids, etc., the scarcity of bacteria being attributed to the acidity of urine and its mechanical flushing effect. Actually, however, the presence of micro-organisms within the urethra is not at all unusual. Helmholz has reported recovery of bacteria at a depth of 5 cm. in the urethras of 50 per cent of normal males.[30] Shackman and Messent report that the normal male urethra may contain "staphylococci, Str. faecalis, diphtheroids, Bact. coli, Str. viridans and bacilli of the Proteus group."[32] Leishman, testing only for E. coli, found it in the urethras of 4 of 25 adult females. Hirshfeld *et al.* found bacteria of some kind in the urethras of about half of 114 normal pregnant women.[31]

One is not justified, then, in assuming that a catheter or other instrument being introduced into the bladder is passing through a bacteria-free passage.

Urine obtained from the bladder and upper urinary passages. There are many reports on cultures of bladder urine and ureteral urine, although most of these deal with patients in whom there had been some reason to suspect presence of urinary tract disease. For obvious reasons the data on normal subjects are insufficient, although several studies have been carried out on normal pregnant women. The techniques of catheterization, of bacteriological procedure have varied considerably, as have the population groups studied. The surprising thing, however, is the frequency with which bacteria have been found. The published reports from various sources appear to indicate that one can expect bacteria in the urine of from 5 to 40 per cent of normal adult people who give no evidence or history of urinary tract disease.[40, 41, 42] According to Schulte[38] the "normal flora" of urine of normal human subjects, arranged in order of frequency is "micrococcus, diphtheroids, strep. faecalis, alpha streptococcus, gamma streptococcus, Staphylococcus albus, E. coli, A. aerogenes and Pseudomonas."

In evaluating these studies the possibility of false positive results due to contamination by organisms introduced into the bladder urine during passage of catheter or cystoscope can never be eliminated positively. Helmholz and Millikin[29] found that even with the most careful technique, including thorough washing of the meatus, they got positive cultures in bladder urine in "one half to two thirds" of normal infants. They concluded: "Chances of contamination are so great that the presence of organisms in the urine does not prove, without further control, that they have come from the kidney." In a compilation of the results of urine cultures of normal pregnant women the incidence of positive results reported by different investigators varied from 13 to 74 per cent.[43] In a similar study bladder urine was said to be "infected" in 43 of 114 antepartum women.[31]

Hundley *et al.*[88] compared bladder urine cultures of 50 normal women and 50 pregnant women. Positive cultures were obtained from 32 of the former and 22 of the latter group. However, E. coli was encountered in only 9 of the 54 positive cultures, most of the organisms found being of doubtful pathogenicity, i.e., staphylococcus albus, streptococcus viridans, diphtheroids, and yeasts.

Studies such as those quoted would have provided more meaningful information if some rough quantitative test had been made. It is probable that up to 1,000 micro-organisms may be found per ml. in normal urine, due to unavoidable contamination from the surface of the urethra during urination or catheterization. In active infection, however, the number of organisms is likely to be from many thousands to millions per ml. of urine. A simple and practical quantitative bacteriological test is to examine a stained smear of the urine. If bacterial cells can easily be found by this method, it is safe to assume that more than 10,000 per ml. are present. Another useful procedure is to dilute the urine, say 1 to 100 and 1 to 100,000, before inoculating culture media. Growth obtained from the higher dilution would be indication of active infection.

THE SOURCE OF BACTERIA WHICH INFECT THE URINARY TRACT

There cannot be much doubt that the origin of the micro-organisms which cause pyelonephritis is in the flora of the intestinal tract. This can confidently be inferred from the bacteriology of pyelonephritis, since coliform bacilli are responsible for about 80 per cent of early, uncomplicated cases. Staphylococci, enterococci, Proteus, and Pseudomonas may also be found, especially in complicated cases of long standing, but these too probably get to the kidney from the bowel. Story[89] compared Proteus strains from urine with Proteus from the same patients' bowel contents and found most pairs from individual patients to be identical. He concluded that autoinfection from the gut is the most frequent mechanism of Proteus urinary infections. Jensen reported that he could lower the incidence of complicating urinary infection following urological operative procedures by first reducing the bacterial content of the intestinal tract with antibiotic therapy.[68]

CONDITIONS AND DISEASES WHICH ARE ASSOCIATED WITH PYELONEPHRITIS

Pyelonephritis of young children. Although exact figures are not available there seems no question about a high incidence of acute pyelonephritis in children between six months and three years of age. It is generally agreed, too, that the disease is commoner in females than males, perhaps 3 : 1. This occurrence of pyelonephritis during the diaper period, when fecal contami-

nation around the urethral meatus is likely to occur, together with the higher incidence in females, has been thought to indicate that the pathway of infection in these cases is via the urethra. Female children would seem to be more susceptible because of the short length of the urethra and greater chance of soiling its meatus. As will be discussed later, this kind of pyelonephritis is very possibly an example of ascending urinary tract infection.

It is generally believed that the pyelonephritis of infants has the same potentialities for chronicity and serious complications as other forms of the disease.[80, 15, 81]

Pyelonephritis of pregnancy. Acute pyelonephritis occurs in about 2 per cent of pregnant women, usually during the second half of pregnancy. It is not an inconsequential infection, probably being an etiologic factor in some cases of toxemia,[61] and certainly being followed by chronic pyelonephritis, with all of its serious potentialities.[61]

Many opinions have been expressed about the cause of the high incidence of pyelonephritis in pregnancy. The most obvious lead is the dilatation of the kidney pelves and ureters, demonstrable in most women after the third month and generally disappearing a few weeks after parturition. The dilatation is more frequent and of greater extent on the right side. It involves the ureter only to the pelvic brim, that portion lying within the true pelvis maintaining normal caliber.

The cause of ureteral dilatation in pregnancy has been discussed extensively. Some have suggested that it is a matter of obstruction by the pregnant uterus, since similar effects have been seen in patients with other large pelvic tumors.[1] The fact that the right ureter seems to be affected more than the left has been attributed to: (a) dextro-rotation of the uterus, (b) the wider angle at which the right ureter crosses the iliac vessels, and (c) protective cushion of the sigmoid colon overlying the left ureter. Another possible cause of the ureteral dilatation which has been proposed is the great hypertrophy of the longitudinal sheath of muscle bundles at the lower end of the ureter.[39] This, however, would not easily explain the greater involvement of the right ureter or the fact that the dilatation is observed only above the pelvic brim. Another possibility is that the ureteral dilatation is part of the atony that affects several smooth muscled structures during pregnancy (uterus, colon, gallbladder). In support of this, Traut and McLane have found diminished amplitude of the ureteral peristaltic wave after the third month of pregnancy.[61] A reasonable conclusion, then, would be that atony of pregnancy, plus obstruction by the gravid uterus and the anatomical factors which render the right ureter more susceptible are all involved in the changes which occur during pregnancy.

Why does infection tend to occur in the kidneys under these circumstances? We must not forget that ureteral dilatation occurs nearly always, but pyelonephritis only once in 50 cases. The available information does not show the bladder urine in normal pregnant women to have more bacteria than the bladder urine of nonpregnant women[33]; the vesical irritability so common in pregnancy is not to be regarded as a manifestation of cystitis. There remain these possibilities: (a) It is said, but without any kind of support, that the constipation often present in pregnancy favors transport of enteric organisms from colon to the kidney via lymphatic anastomoses. The reasons for rejecting the likelihood of this pathway will be considered in detail later. (b) Barksdale[4] reported that he could demonstrate vesicoureteral reflux in some pregnant dogs, but not in normal or post-partum animals. This might facilitate passage of bacteria from the lower to the upper urinary tract. (c) A dormant infection in the kidney may be activated in pregnancy, possibly because of unidentified factors associated with pregnancy, comparable to the higher incidence of poliomyelitis, or possibly because of low-grade obstruction to the drainage of urine. That there is increased hydrostatic pressure in the atonic dilated ureter is indicated by the work of Hundley *et al.*[35] who found that the ureteral and pelvic dilatation could be reduced by allowing an ureteral catheter to remain in place for 48-72 hours. (d) Bacteria which happen to escape from the bowel may be carried to the kidney in the blood, and there find conditions suitable for growth and establishment of a new infection, as outlined under (c).

Diabetes mellitus. Urinary tract infection is commoner in diabetics than in nondiabetics, although precise data on this are lacking. Harrison and Bailey searched for bacilluria and pyuria in 50 patients with diabetes mellitus and in 50 control individuals of comparable age groups. They found a higher incidence of both in the diabetic group.[20] Baldwin and Root discussed 80 cases of upper urinary tract infection in patients with diabetes mellitus observed in one clinic over a 5 year period, 37 of which had terminated in death. They were of the opinion that urinary tract infections in diabetics are more frequent, more protracted, and more serious in nature. They quote statistics based on autopsy examination indicating that 20 per cent of the diabetic individuals show evidence of bacterial infection of the kidney, as compared with 4 per cent of nondiabetic persons.[2]

Necrotizing papillitis, a severe and fulminating form of pyelonephritis is much more likely to occur in the diabetic than the nondiabetic patient. Edmondson *et al.*[9] reported on 50 cases of necrotizing papillitis found in 32,000 autopsies at the Los Angeles County Hospital. Twenty nine of the 50 were encountered among 859 known cases of diabetes mellitus in this

series, whereas only 21 examples were found among the remainder, 1,023 of whom had pyelonephritis. It is generally believed that vascular disease associated with diabetes is an important factor contributing to the greater frequency of renal papillary necrosis in diabetic subjects.

Why should pyelonephritis be more likely to occur in the patient with diabetes mellitus? Possibilities which suggest themselves are: (a) Patients with diabetes are generally believed to be more susceptible to infections, e.g., carbuncles, tuberculosis, mucormycosis, moniliasis. They may have a similar increased susceptibility to kidney infection. (b) Glycosuria may favor the growth of bacteria in the urine. (c) Catheterization is performed frequently in patients with diabetes, in the course of routine work-up and especially in management of patients in severe acidosis.

Obstructive lesions of the urinary tract. The most obvious and probably most important factor in the etiology of pyelonephritis is obstruction to the free flow of urine anywhere between the kidney pelvis and the urethral outlet. The kind of obstructing lesion varies with the age group: congenital anomalies in children, stone, inflammatory edema or stricture in all age groups, and tumors after middle age.

The statistics on the relation between obstructive uropathy and infection are impressive. Campbell[4] estimates that chronic urinary tract infection is 20 times as frequent in children with anomalous urinary tracts as in normal children. Bell's autopsy findings were that the obstructive type of pyelonephritis is about 12 times as frequent as the nonobstructive type.[7] Bell's data also indicate an apparent difference in incidence of infection depending on whether the obstruction is in the ureter (23 to 46 per cent) or in the bladder or urethra (61 to 83 per cent). He suggested that the difference in frequency of infection might be due to the fact that in low obstruction the bladder becomes distended and that this would tend to cause reflux of urine from bladder into ureter. There is, however, another and perhaps better, explanation, namely, that indwelling catheters and cystoscopic treatments are more frequently employed in patients with low obstruction. These therapeutic procedures may themselves be responsible for introducing the infection.

The terms "stagnation" and "stasis" enjoy great favor when the relationship between obstruction and infection is being discussed. The analogy of the "stagnant millpond and the swiftly-running brook" has been drawn, the implication being that growth of bacteria in an inadequately draining reservoir of urine provides a constant source of re-infection to the adjacent tissues of the urinary system. The concept of the evil influence of urinary stagnation is undoubtedly responsible for the common practice of "forcing

fluids" in urinary tract infection. No evidence could be found, however, that this is of curative value, although of course it may have some virtue in reducing dysuria and retarding stone formation. On the other hand, we all have seen many instances where large daily flow of urine has been obtained in patients with incomplete obstruction, without affecting the existing infection, and conversely have seen infections subside quickly after relief of obstruction whether fluids were "forced" or no.

Campbell states that there is some evidence that in the presence of urinary tract obstruction there is perceptible reduction in the phagocytic activity of endothelial cells of renal capillaries.[4] It has not been possible to locate a detailed description of the work on which this statement was based.

It is easy to demonstrate that ureteral obstruction increases susceptibility of the kidney to infection by bacteria which are injected intravenously. The obstructed kidney usually becomes massively infected and progressively destroyed by pyogenic infection whereas the opposite kidney shows no evidence of infection. The best description of this phenomenon is that of Mallory, Crane, and Edwards.[5] These authors noted also that *the progress of the acute infection could be halted simply by removing the ligature from the ureter.*

The relation between urinary obstruction and susceptibility to infection may be only another example of a general phenomenon, that tissues under increased hydrostatic pressure exhibit diminished resistance to pyogenic infection. For example, pyogenic infection is more common in any organ whose excretory duct is obstructed (gallbladder, salivary gland, pilonidal cyst, lacrimal sac, para-nasal sinus, etc.). Several possibilities might be suggested: alteration in blood flow, alteration in lymph flow, diapedesis of leukocytes, efficiency of phagocytosis.

Disturbances in bladder innervation. Many disorders of the nervous system are associated with disturbance in bladder function and urinary tract infection, important examples being multiple sclerosis, poliomyelitis, diabetic neuropathy, tabes dorsalis, and spinal cord injury.

The best information about the sequence of anatomical and functional changes comes from studies of war veterans who sustained spinal cord injury and paraplegia. In special hospitals set up for the care of groups of such patients some helpful observations have been made, although there seem to be no good data on how soon after injury the first sign of kidney infection makes its appearance. Talbot and Bunts[6] report on pyelographic and cystographic studies. In a group of 331 patients with paraplegia, 16 were found to have developed significant hydronephrosis. Cystograms were done on 15 of the 16, and 10 of those were found to have vesico-ureteral

reflux. Thirteen of the 16 patients living at the time of their report had studies of renal function, and 8 of the 13 showed "serious renal impairment." In a later report from the same center[34] Hutch and Bunts discuss the time relationships between spinal cord injury and the appearance of radiographic evidence of uropathy. They found that hydro-ureter and calyectasis increased steadily during the first two years. Functional damage (judged by excretory urography) is not marked for the first three years, but is likely to increase rapidly after that time. Vesico-ureteral reflux also appears and increases after three years, paralleling the loss of renal function. At another center 56 of 238 (23%) paraplegic patients were found to have developed vesico ureteral reflux."

The foregoing observations seem to make a strong case for the importance of ascending infection in the pyelonephritis complicating neurogenic uropathy, the sequence of events being: retention—catheterization—cystitis—vesico ureteral reflux—pyelonephritis.

Iatrogenic urinary tract infection. One of the most important questions that must be asked is how frequently some therapeutic or diagnostic procedure such as catheterization or cystoscopy causes urinary tract infection? Catheterization is one of the commonest procedures in medical practice today. Obstetricians, gynecologists, and urologists unhesitatingly do it as a routine practice before a variety of procedures. It is carried out one or more times in a considerable proportion of postoperative patients. Indwelling catheters are often employed routinely in the management of unconscious patients. Clinical investigators leave catheters in the bladder during certain kinds of renal function test.

Opinions vary greatly on the danger of catheterization. The fact that no one would use an unsterile catheter or fail to make some gesture toward antisepsis in cleaning the area around the meatus proves that some risk is acknowledged, still the very frequency with which the procedure is done indicates that many clinicians believe the danger to be small. At the other extreme there are experienced clinicians who regard the danger of catheterization sufficient to require stringent indications for it. Certainly cystitis developing as a result of one or two postoperative catheterizations is no great rarity. Marple's study furnishes some information on this point.[35] He catheterized 100 female patients on a medical ward service, employing strict aseptic and antiseptic technique, but observed that four of his patients developed urinary infections one to three days later.

In considering this subject we should differentiate between simple catheterization and the indwelling catheter. When the latter is used, the urethra is distended by a foreign body for days or weeks. The response to

this is production of a sheath of mucopurulent exudate around the catheter, providing a splendid medium for growth of micro-organisms. *Infection of the bladder cavity is almost inevitable* under these circumstances,[32] and is not prevented by irrigations or by "prophylactic" antibiotic therapy. As emphasized by Kass,[33] prophylactic antibiotic therapy in such circumstances only serves to ensure that the infection will be caused by organisms not easily affected by available antimicrobial agents.

Simple catheterization does not seem to have all of the dangers associated with the indwelling catheter but, as has already been discussed, it does carry some risk of introducing bacteria into the bladder, either from errors in aseptic and antiseptic techniques or from contamination by bacteria lying in inaccessible parts of the urethra. Garrod *et al.* have some data which they interpret as indicating that catheterization increases the likelihood of enterococcal infection in patients with pre-existing infection.[34]

Scandinavian workers have laid special stress on the danger of catheterization, cystoscopy, etc. Ørskov, by typing Klebsiella strains isolated from urinary infections of patients in one hospital, obtained convincing evidence that cross-infection was a common occurrence.[35] She suspected, but did not prove, that inadequate sterilization of catheters and other urologic instruments was responsible. This view was endorsed by Lindau and Ouchterlony in discussing Ørskov's findings. In a later report, however, Ørskov suggests that indwelling catheters may become fecally contaminated by the hands of the individual patients.[36] Jensen[37] reports that every one of 14 patients with previously sterile urine had bacteria in the urine within 48 hours "after operation or large instrumental interference." Jonsson and Erlanson stated that "practically all" patients with prostatic disease or bladder tumor, requiring catheterization or indwelling catheter, became infected during their stay in hospital, whereas the incidence of infection in patients with disorders not requiring catheterization, such as malformations and urinary calculi, was comparatively low.[38] Similar opinion is voiced by Halkier *et al.*[39] This is in agreement with Bell's finding,[1] mentioned previously, that pyelonephritis is commoner in patients with obstruction to bladder emptying than in patients with ureteral obstruction.

One further danger of urethral instrumentation is that it may produce a transient bacteremia. Clinicians have known since the last century that the passage of catheters and sounds might be followed by fever. It is now known that the fever is a manifestation of bacteremia, the mechanism doubtless being sudden increase in tension of inflamed tissues around the urethra causing lymph containing bacteria to flow in the direction of the blood stream. Powers summarized the literature on this subject in 1930, including some interesting quotations on "catheter fever" from the older literature.[40]

In view of the possibility that infection of the kidneys may take place via the blood stream, *the bacteremia of urethral instrumentation probably represents one of the ways in which infection is transferred from lower to upper urinary tract.*

Hereditary pyelonephritis. One example of high familial incidence of pyelonephritis has been observed among a kindred in Utah.[70] About 50 members had renal disease similar to the entity described by Weiss and Parker,[68] but progressive insufficiency developed only in the males affected. This observation, although of interest, does not provide additional clues in respect to the problems under consideration here.

SOME QUESTIONABLE ASSOCIATIONS

Acute infectious diseases in children. Statements are occasionally encountered to the effect that children are liable to pyelonephritis following acute respiratory infection, otitis media, etc. There is actually no convincing evidence of any such relationship, and indeed when we consider that children average half a dozen acute respiratory illnesses per year, it would be a practical impossibility to obtain proof that the relationship exists.

Diarrheal disease. Schwartz, in 1918, described an extraordinary frequency of urethral and bladder contamination in infants, with diarrhea, especially in females.[86] Since that time there have been numerous statements about an increased incidence of urinary tract infection following diarrheal disease in children. No conclusive data on this have yet been provided, and the possible relationship seems unproved as of now. It is interesting to note, however, that Leishman[50] found 19 urine cultures positive for E. coli among 30 women with diarrheal disease, and only one positive among 16 men with diarrheal disease. None of those patients had pyuria or symptoms of urinary infection.

Constipation. The impression that constipation favors urinary tract infection seems to have been widely entertained, but a definite correlation has not been demonstrated, indeed, one wonders how it ever could be, in view of the frequency and indefiniteness of constipation as a symptom. A negative correlation was obtained by Leishman, who performed urine cultures on a group of 14 men and 40 women, all with "obstinate constipation." The proportion of positive cultures was almost identical with that in his control groups. It would seem that for the present we should omit constipation from the list of factors in the pathogenesis of pyelonephritis.

Birth trauma. The view that the relaxations which may follow birth trauma predispose to urinary tract infection seems to be only a clinical

impression, almost impossible to substantiate or to disentangle from such factors as catheterization, residuals of pyelonephritis of pregnancy, etc. In Marple's study of 100 female medical patients[6] no evidence of a higher incidence of positive urine cultures associated with birth trauma was observed.

"Honeymoon cystitis and pyelitis." Gynecologists agree that symptoms of cystitis are not rare in women during the first few days of marriage. This is ascribed in some way to the trauma of sexual intercourse. It is not an easily studied entity, and the symptoms are probably not always due to infection. No evidence whatever was found to support the concept of "honeymoon pyelitis," and this term should be abandoned.

THE POSSIBLE PATHWAYS BY WHICH BACTERIA REACH THE KIDNEY

Let us now consider some of the evidence pertaining to the oft-debated problem: by what route do bacteria travel from the intestinal tract to the substance of the kidney? Medical literature contains many strong statements of opinion, but there is still nothing conclusive. Writers often seem to have drawn unwarranted conclusions based upon a set of animal experiments or some clinical observation on one form of pyelonephritis. To cite a recent example: Barnard, Story, and Root[7] state that 51 of 52 patients who were referred to a special clinic for urinary tract infections in diabetics were women. They seem to regard this fact alone as reliable evidence that the initial event in urinary tract infection is urethral contamination. Cabot and Crabtree[13] in their masterful summary of the problem in 1916 came out forcefully in favor of the hematogenous pathway of infection and indulged in some sarcasm regarding other points of view; yet they fell back on the following statement to explain the higher incidence in women: "In the adult female, we think, there can be no doubt that the large intestines are more favorable to the occurrence of bacilluria than in the male. There is as yet no adequate explanation of the greater frequency of these infections in the female child, but we think that existing evidence points to the view that this explanation will be found, not in the anatomical peculiarities of the female urethra, but in the anatomical peculiarities of the female intestine."

Infection of the kidney by way of the lymphatics.

(a) *From the intestine directly to the kidney.* Postulation of the importance of this pathway of infection has been popular since the work of Franke[20] in 1910. He demonstrated lymphatic connections between the appendix and cecum and the right kidney. This fact has been cited repeatedly as a possible explanation for the greater frequency of right-sided upper urinary tract infection. However, it should be noted that Franke

searched for, but failed to find, any similar lymphatic connection between the left kidney and the colon. While the right kidney is involved somewhat more frequently than the left, anatomical factors already discussed may account for the difference which is noted in pregnancy.

If we assume that bacteria do sometimes pass from the bowel into the intestinal lymphatics, what is the likelihood that they will travel to the kidney in preference to the usual pathway into the para aortic system and eventually into the thoracic duct and thence to the circulating blood? The mere demonstration that dye injected under pressure exposed channels of communication between the ascending colon and the right kidney does not prove that lymph from the bowel would ordinarily travel by such a pathway, and certainly does not explain left sided or bilateral disease. Where lymphatic obstruction exists, as in filariasis, chyle may appear in the urine, but this is certainly a rare event.

(b) *From the lower urinary tract.* In many instances there appears to be infection in the bladder, urethra, and para-urethral structures for a variable period of time before there is a spread to the upper urinary tract. Several writers have suggested, therefore, that the course of events is first cystitis, then migration of bacteria into the rich lymphatic network of the lower urinary tract, and by peri-ureteral lymphatic vessels to the kidneys.

The weak point in this hypothesis lies in the fact that direct lymphatic communication between the lower and upper urinary tracts has not been demonstrated convincingly. Sweet and Stewart[28] and Eisendrath and Kahn[10] believed that they had shown its existence in animals, but their principal evidence was lymphocytic infiltration in the outer coats of the ureters following inoculation of bacterial culture into the bladder. As pointed out by Bell, these changes may simply be due to ureteritis.[7] Eisendrath and Kahn made the wholly unwarranted assumption that the possibility of blood stream transfer of bacteria from bladder to kidney was ruled out by the fact that heart's blood culture was negative at the time of sacrifice of the animal. Winsbury-White[29] in a most unconvincing, but frequently quoted, piece of work claimed that he had demonstrated passage of India ink particles from the lower to the upper urinary tract via lymphatic vessels in some animals. He gave so few details that one cannot now evaluate the possible loopholes or soundness of his work.

On anatomical grounds it seems unlikely that there are direct lymphatic communications between the lower and upper urinary tracts, because there are no parallel channels of blood supply. The general rule is that lymphatic drainage of a region follows its blood supply and venous drainage. The arteries and veins of the urinary tract are segmentally distributed, even at

different levels of the ureters. It would be surprising, then, to find that the lymphatics run upward from bladder to kidneys around the ureters. The spermatic and ovarian veins are in separate fascial sheaths having no close connection with the urethra or bladder. The excellent study of MacKenzie and Wallace[10] shows in fact that in rabbits dye particles injected into the uterine cervix, bladder wall, and ureter travel not toward the kidney but into the common iliac glands, thence upward along the aorta to the thoracic duct. Their findings contradict Winsbury-White's.

It appears unlikely then, that the postulated pathway from lower to upper urinary tract via the lymphatics exists at all.

Hematogenous. The principal points for and against the blood stream as the principal pathway of kidney infection are as follows:

(a) No one questions that coccal infections of the kidney develop as a result of hematogenous dissemination. Oftentimes no other anatomical possibility exists, as, for example, when renal cortical abscess develops subsequent to carbuncle of the neck.

(b) It is usually assumed, although without much factual proof, that enteric bacteria must occasionally escape from the intestinal canal of the human being, and be carried to the blood, via lymphatics. This would provide opportunity for some of them to reach the substance of the kidney, where under favorable circumstances they might be able to establish infection. Actually, coliform bacteria are not common "contaminants" of blood cultures of human beings, and their presence in the blood is seldom noted in the absence of definite coliform infection in the urinary tract or elsewhere. Furthermore, many experiments in animals have shown that only a small proportion of bacteria which are injected into the blood stream are trapped by the kidneys. One might ask why, if enteric organisms are frequently disseminated in the blood, the principal tissues to suffer are the kidneys?

For the concept of infection by the path: normal intestinal tract → lymphatics → blood → kidney—one would like to have a little more evidence that enteric bacteria do in fact get into the blood stream occasionally in healthy human beings.

(c) It can be accepted as proved beyond question that bacteria may reach the blood stream following instrumentation of the urethra. Bacteremia has been found in a significant proportion of cases immediately after the passage of a sound or cystoscope.[7] This having been proved, one is justified in assuming that instrumentation may not always be required, and that bacteremia may occur in the course of a lower urinary tract infection as a result of normal lymphatic drainage and perhaps augmented by such acts as micturition or straining to defecate.

On the whole, then, there can be little question that bacteria sometimes reach the kidney by the path: infected bladder or urethra—lymphatics—blood—kidney.

(d) It has been amply demonstrated that kidney infection can be induced in animals by bacteria injected into the blood stream. In the case of staphylococci[37] and C. renale[32] this occurs in previously normal kidneys. In the case of coliform bacteria, however, in order to obtain kidney infection in a high proportion of experimental animals it is necessary to injure the kidney in some way first. The usual method employed is to obstruct the ureter.[4] The same effect can be obtained, however, by trauma to the kidney before inoculating the bacteria.[9, 10]

(e) Some writers have pointed to the cortical distribution of lesions in early pyelonephritis as evidence favoring the hematogenous pathway of infection.[23, 40] However, Kennedy, in what appears to be an important contribution,[20] says that it is futile to attempt to decide the portal of entry on the basis of distribution of lesions in the human kidney. He had the opportunity to study the organs of four children who succumbed within a few days after the inception of acute pyelonephritis and found the lesions to be widespread in all of them. He carried out animal experiments in which the bacteria were inoculated intra-ureterally or intravenously and found that by the end of 48-72 hours the lesions in the two groups were indistinguishable.

Ascending infection. Cabot and Crabtree, before detailing a series of objections to it, remarked in 1916 that "the theory that infections reach the kidney by direct extension from the lower urinary passages has acquired the dignity of old age and has been a widely held and attractive theory because of its extreme anatomic simplicity."[18] Despite the arguments presented by these writers the theory is still an attractive one, supported by a variety of clinical and experimental observations.

The main question in connection with the ascending pathway revolves about the possibility of movement of bacteria out of the bladder into the ureters and thence to the kidneys. It is generally accepted that bacteria gain entry to the bladder most frequently by way of the urethra. We should, therefore, consider at this time the evidence on vesico-ureteral and ureteral reflux.

Vesico-ureteral reflux. There is lack of agreement about the frequency with which reflux occurs in normal individuals. Many observers are impressed with the mechanical excellence of the vesico-ureteral valve, and believe that it is rarely if ever incompetent. At autopsy the ureteral orifices are seldom found widely patent even in patients who have had obstruction to bladder emptying for some time. One urologist states that in 722 cysto-

grams reflux was never observed except in the presence of some other uropathy.[4] Another, however, says that he was able to demonstrate reflux in 2 of 43 children without known urologic disease.[22] Another reports that reflux was encountered in 3 of 10 normal children and 1 of an unstated number of normal adults. The various pieces of evidence on this subject up to 1944 were reviewed by Prather.[23]

The employment of the "delayed" cystogram [12] seems to be an improved way of demonstrating reflux when it is present. In this technique the bladder is filled with radio-opaque material, a picture is taken, the patient is allowed to get up and move about; a second exposure is made after 30 to 60 minutes. In some instances the second examination reveals reflux when the one done immediately after filling the bladder had not.

Considering that cystography is our only method of testing for reflux in the human being and that it will only disclose a comparatively gross spilling sufficient to be visible in the x-ray film, it seems reasonable to suspect that escape of small quantities of urine from bladder into ureter may occur more frequently than can be detected, even in persons with normal urinary tracts. For example, greater distension of the bladder can be achieved when it is gradually filled with urine than when fluid is rapidly introduced through the urethra, as is done in cystography. The mechanical effectiveness of the valve undoubtedly depends on the long oblique course which the ureter takes through the bladder wall, but when the bladder becomes greatly distended, the wall thins out, and the ureter must take a nearly perpendicular course.

Reflux into the ureters is a fairly common finding in patients who have chronic urinary tract infection, even when the appearance of the ureteral orifices as seen through the cystoscope is normal. Bumpus reviewed the records of 1,036 patients on whom cystography had been performed.[1] He found that chronic reflux from bladder to ureters was demonstrated in 8 per cent of them. He suggested that reflux probably occurs because the intravesical portion of the ureter is involved in inflammation and changes from its normal easily compressible state to that of a semi-rigid tube. This appears to be a plausible explanation of the high incidence of reflux in chronic urinary tract infection, and is perhaps the mechanism responsible for extension of infection from the bladder to the kidneys in patients with long-standing bladder infection, e.g., in paraplegic individuals with indwelling catheters. One other possibility might conceivably play some part in this relationship between urinary tract infection and vesico-ureteral reflux: individuals who already have incompetent valves may develop upper tract infection because of this defective barrier.

Mention has already been made of the possibility that reflux may occur temporarily in pregnancy. This suggestion, based upon observations in dogs, has not been substantiated in the case of human beings; in fact, in one small series of cases tested for it, reflux was not found.[42]

Studies on animals show that vesico-ureteral reflux does occur when the bladder is greatly distended, and the workers who have made these studies have concluded that this probably occurs in the human being and may be a significant factor in the etiology of pyelonephritis.[25, 28]*

Ureteral reflux. Among the arguments against ascending infection are statements that spread of infection upward via the ureter would be prevented by the effects of gravity and ureteral peristalsis. These arguments are not very convincing. Gravity would not oppose flow toward the kidney when the individual is lying in the recumbent position. A drop of fluid can squirt fast and far through a collapsed tube, as is observed, for example, when the last few drops of urine are expelled through the urethra at the end of micturition, or in the passage of dyes injected into lymphatic vessels. Forces which could provide this propulsion up the ureter might be contraction of the bladder or of the abdominal muscles during movement, coughing and straining. In cystography, once the radio-opaque material gets by the uretero-vesical junction it usually travels up to the renal pelvis in a few seconds.

As for the matter of peristalsis in the ureter, not enough information is available. The excellent acute experiments of Lapides[39] indicate that these structures are more or less autonomous, not affected by a variety of pharmacological agents or the autonomic nervous system. The only stimulus which he found to be effective in stimulating forward peristalsis was the entry of fluid from above. Reverse peristalsis has been observed, however, in the ureters of experimental animals[29]; furthermore, the force of ureteral peristalsis can be overcome if the pressure from the bladder is great enough.[37] Reverse peristalsis or retrograde flow must occur at times in the human ureter. Stones have been observed to move from the lower ureter back up to the kidney pelvis in successive x-ray examinations. Foreign bodies introduced into the bladder have been recovered from the kidney pelvis,[32] and solid fecal material has appeared in the nephrectomy drainage of patients with uretero-colonic anastomoses.[2] The frequency of massive

* Dr. Charles Kleeman has observed what appeared to be instances of this in two healthy young physicians serving as subjects for experiments involving severe diuresis. In attempting to empty the bladder only at specified intervals an uncomfortable overfilling had developed. Both subjects described sudden onset of severe pain in the flank and kidney region, promptly relieved by emptying the bladder. It seems probable these are instances of vesico-ureteral reflux due to overdistension of the bladder.

infection of the kidney in patients with uretero-colonic anastomoses is further proof that infection can easily reach the kidney via the ureter once the safeguard of a normal uretero-vesical valve has been sacrificed.

Let us turn back now to a consideration of the evidence for and against the ascending pathway in the different conditions under which pyelonephritis occurs.

(a) The arguments against ascending infection are all levelled against the likelihood of spread past the vesico-ureteral valves and up the ureters, since it has always been more or less taken for granted that the portal of entry in cystitis is by way of the urethra.

(b) We have already noted the greater frequency of acute pyelonephritis in female children and the opinions that it is related to greater chance of fecal soiling of the urethral meatus during the "diaper" period. The greater frequency of cystitis in adult females has also been noted. A related point is that catheterization is carried out more frequently in females than in males (except possibly in the older age groups). That catheterization carries some risk of cystitis cannot be denied. Yet women are often catheterized "routinely" in obstetrical and gynecological procedures, and much more frequently than males to obtain urine for culture or microscopic examination.

(c) While the manifestations of acute pyelonephritis sometimes come on without preceding symptoms of urinary tract infection, e.g., in pregnancy and in young children, we see many instances where cystitis precedes evidence of kidney infection for a considerable period of time, e.g., in neuropathic bladders and in prostatic disease. Here it is almost certain that *infection spreads from the bladder to the kidney*. From a practical standpoint this is perhaps the most important thing to recognize: that bladder infection, not very serious in itself, is dangerous as a precursor of pyelonephritis. Whether the route from bladder to kidney is via the ureter or the lymphatics and blood is less important.

(e) The ascending route appears less obviously the pathway of infection in pyelonephritis of pregnancy and the other instances where signs of acute renal infection appear abruptly in a person who has had no previous urinary disorder and whose urinary tract appears normal. In these cases symptoms and signs of cystitis are absent or minimal, and if present at all, they seem to be secondary to the kidney infection. To explain such cases on the basis of ascending infection one would have to postulate: (1) Entry of bacteria into the bladder, despite the bactericidal action of the urethral mucosa and the mechanical barrier of the bladder sphincter. Admitting that all of the information we have on the bacteriology of "bladder urine" is subject to the error of possible contamination by bacteria picked up from the

lining of the urethra, it must be acknowledged that all workers have reported presence of bacteria in a formidable proportion of normal individuals. Even if we disregard organisms of doubtful pathogenicity it is perhaps significant that the incidence of E. coli in the "bladder urine" of antepartum women is 6 per cent or higher.[40, 53] Yet these are single samples; perhaps repeated examination throughout the course of pregnancy would reveal E. coli in the bladder at one time or another in a much higher proportion of women. (ii) Multiplication of bacteria in the bladder, without pyuria or dysuria. This is not difficult to conceive of, and David's work indicates that it can take place in dogs.[19] He found that instillation of E. coli into the unobstructed, nontraumatized bladder did not cause development of a cellular exudate, but that organisms could be isolated from the bladder urine for as long as a month afterward. (iii) Passage through or around the uretero-vesical valve. Here we cannot fall back on chronic inflammation and stiffening of the intra-vesical portion of the ureter with reflux, or on chronic bladder distension thinning out the valve. Does this happen now and then when due to some circumstance an individual does not empty the bladder until it has been uncomfortably distended for a period of time? Or are there frequent small refluxes, so minute that they could never be detected by cystography? Does the demonstrated abnormality in function and tone of the ureters during pregnancy also include a small proportion of women liable to vesico-ureteral reflux? (iv) Progression up the ureter to the kidney. In a preceding section this subject was discussed, and some reasons were given for stating that ureteral peristalsis and gravity do not appear to be adequate safeguards against this possibility.

Although the passage of bacteria from urethral meatus up to the kidney via the urinary passages is not the matter of "delightful simplicity" it appears to be at first glance, especially in persons without demonstrable disease of the urinary tract, it is certainly within the bounds of reasonable possibility.

SUMMARY AND CONCLUSIONS

Information derived from clinical observations or from animal experiments which may shed light on factors of importance in the pathogenesis of pyelonephritis has been evaluated.

Pyelonephritis has certain peculiarities as an infectious disease. Although the causative bacteria are regarded as extracellular parasites which ordinarily provoke a purulent exudate, they seem capable in the kidney of establishing an infection of extraordinary chronicity. Although most of the causative bacteria are susceptible to one or more potent chemotherapeutic

agents, the results of drug therapy are surprisingly poor, a fact not readily explainable on the basis of such factors as presence of pus, inadequate blood supply, or poor surface for phagocytosis. Lastly there is no good explanation for the well-recognized tendency of urinary obstruction to enhance the infectious process. Further study of these problems is needed.

Evaluation of clinical data on urinary tract infection is hampered by certain sources of error: (i) Lack of quantitative data on urine cultures due to confusion as to the existence of infection or "contamination" by harmless flora of the urethral channel. (ii) There is often no simple and reliable method of differentiating between infection of the lower and upper urinary tracts. (iii) "Bladder urine" obtained by catheter may give a falsely positive culture because it is impossible to sterilize the urethral canal before passing a catheter through it. (iv) The chronicity of pyelonephritis, with remission and exacerbations, renders recognition of the conditions associated with first attacks difficult in many instances.

Acute pyelonephritis occurs with unusual frequency in early childhood and in pregnancy.

Diseases in which there is an unusually high incidence of pyelonephritis include: (i) those with interference with bladder innervation, (ii) those with obstruction to urinary flow, and (iii) diabetes mellitus.

Clinical impressions that it is a complication of respiratory or intestinal inflammation, constipation, or birth trauma lack convincing substantiation.

The part played by catheterization and other instrumentation in causing infection of the urinary tract is discussed. There is substantial evidence that this is one of the major factors in the etiology of urinary tract infection at the present time. There is, however, a regrettable lack of factual information on these problems, e.g., the frequency with which simple catheterization causes cystitis, and, in turn, the frequency with which cystitis leads to pyelonephritis.

The theories offered as to the pathways by which enteric bacteria travel from the bowel to the kidney are discussed. The conclusion is reached that there is little support for the idea that bacteria travel directly from the intestine to the kidney in lymphatic vessels or that they are carried from the lower to the upper urinary tract in lymphatic vessels. It is possible, indeed probable, that they sometimes pass from the bowel or the lower urinary tract into the lymphatic system and thence into the blood stream and to the kidney. Instrumentation of an infected lower tract is known to cause bacteremia.

"Ascending" infection, via the urinary passages, is almost certainly the method by which pyelonephritis occurs in some conditions, notably neuro-

genic bladder disturbances and in diseases such as prostatic hypertrophy, where the bladder is chronically distended.

Evidence on the mechanism and occurrence of vesico-ureteral and ureteral reflux is considered.

Stress is laid on the importance of bacterial contamination of the urinary bladder as a factor leading to the development of pyelonephritis. This appears to be a subject deserving of careful consideration, since it is undoubtedly easier to prevent or to treat than pyelonephritis.

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SERUM BUTANOL-EXTRACTABLE IODINES IN ABORTIONS‡

INTRODUCTION

Previous investigations suggest that certain women when pregnant are deficient in circulating thyroid hormone and that this deficiency is associated with a tendency to abortion. Using the serum iodine as an index of thyroid activity, Heinemann *et al.*[18] in 1948 carried out an investigation of the serum precipitable iodine (SPI) in pregnant women. The SPIs of approximately 75 normal non-pregnant women ranged from 3.8 to 7.8 gamma per cent with a mean of 5.6 ± 1.3 gamma per cent. The concentrations of SPI in 29 normal pregnant women ranged between 6.2 and 11.2 gamma per cent with an average of 8.4 gamma per cent. An increase was noted as early as the third week of pregnancy and subsided within two weeks after delivery. These values, which otherwise would be indicative of hyperthyroidism, are physiological for pregnancy[2, 10, 25, 30, 33, 34] with the rise antedating the elevation of the basal metabolic rate. In some patients who threatened to abort or who actually aborted, the SPI ranged between 2.8 and 5.8 gamma per cent. Thus it appeared that if the SPI did not rise before the sixteenth week of gestation, abortion was likely to occur. Some evidence was presented that the administration of desiccated thyroid might diminish the tendency to abortion. Results of further investigations[19, 25, 30] were similar, but that an abortion regularly follows low concentration of circulating thyroxine-like iodine compounds has not been conclusively demonstrated.

With regard to the effect of overactivity of the thyroid on the pregnant state, Heinemann *et al.*[18] noted that hyperthyroidism in four of the pregnant women in their study was easily controlled and pregnancy maintained with Lugol's solution alone or in conjunction with small amounts of thiourea. Peters *et al.*[26] are also of the opinion that the course of pregnancy is normal

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in the hyperthyroid woman adequately treated medically. This opinion is generally accepted at the present time.

The present paper deals with the butanol extractable iodines (BEIs) of 26 women who aborted and of two patients who threatened to abort. Earlier work from this laboratory involved SPIs, which are sometimes spuriously high after ingestion of inorganic iodine, but included only a few measurements of thyroxine like iodine compounds in serum of pregnant women and of those who aborted.

METHOD AND MATERIALS

The subjects were 28 women admitted on the gynecological service of the Grace-New Haven Community Hospital because they had aborted or threatened to abort. Two of the patients did not abort, and the BEIs of these women were determined when they were threatening to do so. All except one of the 26 abortions were spontaneous. The ages of these 28 subjects ranged from 18 to 41 years.

Upon admission, physical examinations and detailed histories were made with special reference to thyroid abnormality, previous obstetrical performance, and the occurrence of vaginal bleeding and cramps earlier in the course of the present pregnancy. The latter symptoms were investigated to determine the possibility of an abortion having actually occurred prior to hospitalization. This was also checked by comparing the uterine size as determined clinically with the duration of pregnancy calculated from the date of the last menstrual period. In thirteen of the patients, urinary chorionic gonadotrophins, determined at some time during the course of pregnancy or soon after its termination, were compared with the BEIs. It has been shown that if the gonadotrophin value is less than 3,000 IU/24 hours, abortion is inevitable.[8] The reports from the pathological laboratory were also scrutinized.

Venous blood was drawn as soon after the abortion as possible or taken the following morning before breakfast. The BEIs determined from these samples represent the concentrations found at abortion, although it is difficult to date clinically the precise time when the fetus was no longer viable. For 19 women a follow-up BEI was measured from 5 to 8½ weeks after the recognition of abortion to establish the non-pregnant values. The usual strict precautions against contamination were taken. None of the subjects had had salpingograms or other diagnostic tests employing iodine-containing compounds (e.g., telepaque, terredex, urokon, itrumil, priodax, diodrast, lipiodol) or any other substances (e.g., mercurials) known to affect BEI values.[15, 20, 22] Serum was obtained from blood collected under oil in tubes and allowed to clot before being centrifuged. BEIs were determined according to the method of Man *et al.*[20] with a maximum deviation of 1.0 gamma per cent between the duplicate aliquots.

RESULTS

A complete record of the BEIs of the patients in this study is given in Table 1. The first three exceptional patients could not be classified in the three major groups listed below. Eleven women, patients 4 through 14, had at abortion BEIs (column 4) within the normal range for pregnancy as

TABLE I. BEI's OF PATIENTS IN THIS STUDY

	Patient no.	GNHOH no.	Age	Time of BEI after abortion	BEI gamma %	Duration of pregnancy in weeks calculated from LMP	Uterine size	Days after abortion	BEI gamma %
Woman who aborted with a BEI above the normal range for pregnancy									
	§1	C62753	33	20 hours	15.3	9	10	8	14.3
Women who did not abort spontaneously									
	*2	40-34-52	27	*	7.3	17	16	...	...
●	3	B88603	27	*	6.4	*	20	7	7.1
Women who aborted but whose BEIs were within the normal range for pregnancy									
●	4	41-29-25	22	2 hours	7.6	11½	16	...	...
●	5	A35044	21	16 hours	7.0	14½	14	39	4.7
●	6	A49573	29	14 hours	7.0	11	12	44	3.4
	7	41-62-51	24	9 hours	7.0	12	12	...	...
	8	B88917	26	9 hours	7.8	17	22	52	5.1
	9	A59079	36	?	6.5	10	8	...	...
●	10	A65250	18	3 days before	6.3	10	8	56	3.9
●	11	C3765	25	20 hours	6.2	11	8-12	42	3.9
●	12	B29028	24	24 hours	6.1	12	8	41	3.8
	13	41-70-44	33	?	6.4	14	10	84	4.3
▲	†14	A67045	31	2 hours	4.8	6	6	9	5.4
								67	6.2
Women who aborted but whose BEIs were at the minimum for normal pregnancy									
▲	*15	B21311	22	?	5.5	13	8	50	3.9
●	16	C11611	41	16 hours	5.4	8	8	...	...
●	17	41-70-30	27	18 hours	5.4	9	8	38	3.8
	18	39-56-66	41	?	5.6	12½	8	88	3.7
Women who aborted but whose BEIs were below the normal range for pregnancy									
▲	19	41-50-11	23	10 hours	4.0	10-12	10-12	...	...
▲	20	C55446	28	30 hours ?	3.9	7	8	39	3.5
●	21	A44802	37	4 hours	4.9	17	16	...	...
●	22	A47717	21	3 hours	3.9	9	8	51	3.4
	23	B95423	21	week before	3.5	9	10	53	6.2
	24	37-28-92	36	7 hours	4.1	10½	12	37	4.0
	†25	C62508	26	20 hours ?	4.9	9½ *	8	30	5.9
	26	36-76-29	28	10 hours	5.2	17	8-10	49	6.1
●	27	36-72-68	27	6 hours	5.4	15½	16	48	4.5
	28	C43801	32	12 hours before	3.5	10	10	80	3.4

* Receiving 60 mg. desiccated thyroid daily. Still pregnant.
† Receiving 90 mg. desiccated thyroid daily.
‡ Hyperthyroid receiving Lugol's solution.
§ Patient with BEI of 15.3 gamma per cent at abortion who received one grain thyroid daily for one week.

The obstetricians frequently cannot date the time when the fetus is no longer viable. The time interval in the next to the last column may be longer than calculated but in no instance would be less than the estimated interval after abortion.

* Five weeks after this BEI, Cæsarian section was performed for central placenta praevia.

described later. The next four women, patients 15 through 19, had borderline BEIs for normal pregnancy, while the last 10 patients had abnormally low BEIs.

All patients were clinically euthyroid at the time of abortion, although five designated by asterisks in the table cannot be classified as absolutely euthyroid because they were taking or had received desiccated thyroid or Lugol's solution. Patient 1, who had a greatly elevated BEI or 15.3 gamma per cent at abortion, refused to return for an adequate clinical evaluation of her thyroid status and may have been thyrotoxic. She had received 1½ grains of thyroid for a week one month before hospitalization because of vaginal bleeding. Patient 14 with a BEI of 4.8 gamma per cent was a known hyperthyroid patient on Lugol's solution at the time of abortion. Patient 2 who had received 1 grain of thyroid daily for the preceding six months had a serum iodine of 7.3 gamma per cent and did not abort. Patients 7 and 15 with BEIs of 7.0 and 5.5 gamma per cent had been taking 1½ and 1 grains, respectively, every day for the preceding year.

In Figure 1 the BEIs at the time of abortion (Table 1, column 4) of 26 women who miscarried are plotted in relation to the duration of gestation calculated from the onset of the last normal menstrual period. Referring to the graph the two horizontal broken lines represent the normal range of serum butanol extractable iodine of human adults, 3.2 to 6.4 gamma per cent.[16] Unpublished data by Man[17] are used in defining the normal ranges of BEI values of women with uncomplicated pregnancies at various intervals throughout their gestation. The lower limit of normal by the eighth week was found to be 5.5 gamma per cent. Values between 5.5 and 7.6 gamma per cent at the tenth week were compatible with normal pregnancy. At about the tenth week the average BEI rises to around 7 gamma per cent and seems to remain at this level without much fluctuation until pregnancy is terminated. These data are derived from only 88 BEIs of 21 patients. They are, however, essentially in agreement with many previous SPI determinations in pregnancy.[11-16] The solid line depicts the average of these iodine values, showing the rather rapid rise from the fifth to the eighth week. No values have been measured before the fifth week, so that the broken line connecting the concentration at this time to the non-pregnant mean value of 4.7 gamma per cent of euthyroid adults[16] does not represent values expected in the interval but is included for continuity.

In this graph six of the 26 BEIs of the women who aborted (patients 1 and 4 through 8 in Table 1) were above, while those for 20 of the females (patients 9 through 28 in the table) lay below the average value for normal pregnancy. The last ten patients (numbers 19 through 28 in Table 1) had

BEIs which were abnormally low. Fifteen of the 20 women had BEIs close enough to the average line to be included within the range for normal pregnancy. Five of the six women with BEIs above the average and six of the 20 females (patients 9 through 14) with BEIs below the average had concentrations considered normal in that they fall within the physiological range for that period of pregnancy. The durations of their pregnancies

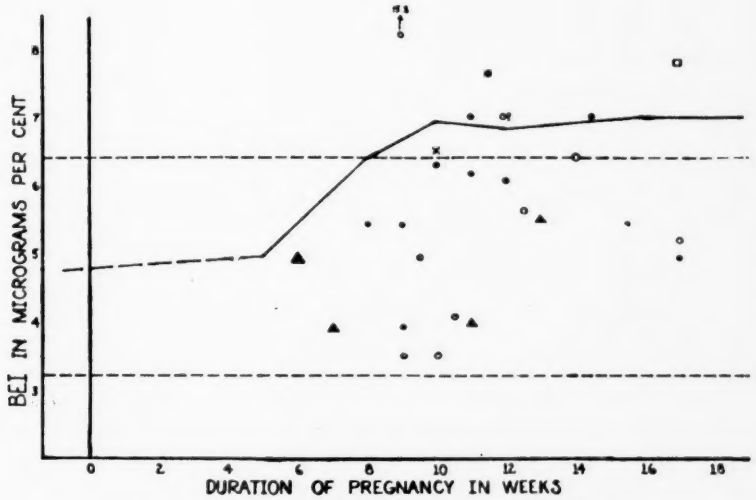

FIGURE 1 - BEI OF PATIENTS WHO ABORTED

LEGEND
- ▲ PRIMIGRAVIDA
- □ HYDATIDIFORM MOLE
- × SELF-INDUCED ABORTION
- ● MULTIGRAVIDA WITHOUT PREVIOUS ABORTION
- ○ MULTIGRAVIDA WITH PREVIOUS ABORTION
- Of MULTIGRAVIDA WITH QUESTIONABLE PREVIOUS ABORTION

ranged from the sixth to the fourteenth week, and the BEIs from 4.8 to 6.5 gamma per cent. Four additional women (patients 15 through 18) with pregnancies from the eighth to the thirteenth week had values of 5.5 ± 0.1 gamma per cent which is borderline normal or slightly low.

Of the 11 patients in Figure 1 with BEIs definitely in the normal range, the cause for abortion is evident in two. The abortion of patient 9 was self-induced, while that of 8 was attributed to a hydatidiform mole. Of the remaining nine patients in the group, four (numbers 4 through 7) had

values above average ranging from 7.0 to 7.6 gamma per cent. The other five women (numbers 10 through 14) with BEIs varying from 4.8 to 6.4 gamma per cent had values slightly less than the average but within the normal range for their respective durations of pregnancy. All of these nine BEIs, high or adequate for normal pregnancy, indicate that a satisfactory concentration of circulating thyroxine-like iodine by itself does not insure a successful gestation. For the 10 patients plotted in Figure 1 who constituted the last group of Table I the expected physiological increase in BEI did not occur. Their iodines from 3.5 to 5.4 gamma per cent are abnormally low for the period of pregnancy at which they were measured.

The time relationship between the abortion and the drawing of blood for the BEI can only be a very rough estimation, for the exact time at which the abortion actually occurred in most of the cases is uncertain. For example, number 11 began to bleed spontaneously three weeks prior to hospitalization and continued intermittently until cramps and more profuse hemorrhage precipitated admission. Various other subjects had spotting at some time during the course of gestation. The criterion of the time of abortion used in this study is the appearance of moderate to severe vaginal bleeding and/or cramps with or without the passage of tissue. Whether a missed abortion had occurred earlier may be judged by comparing in columns 5 and 6 of the table the duration of pregnancy as determined from the last menstrual period and clinically from the size of the uterus. Nineteen patients who aborted show fairly close correlation in this respect. There are seven patients—numbers 4, 8, 12, 13, 15, 18, and 26—for whom there is a significant discrepancy in the duration of pregnancy as determined by these two methods. Number 8 exhibited a larger uterus than expected for her length of gestation, which might be explained by the hydatidiform mole. The larger than normal uterine size of number 4 might be based on an erroneous date of her last menstrual period because an episode of bleeding after conception might have occurred. Her gonadotrophic titer immediately after abortion was between 23,000 and 58,000 IU/24 hours, concentrations not at all suggestive of a hydatidiform mole. Neither was there evidence of multiple pregnancy nor of hydramnios. In the remaining five cases mentioned briefly below, the uterus was smaller than expected, indicating the occurrence of an earlier retained abortion, error in clinical judgment in estimating the uterine size, or inaccuracy in the date of the last menstrual period. There was nothing in the history of number 12 to suggest that an intrauterine death of the fetus might have occurred earlier. Number 26 with a similar history had a benign pregnancy until one week prior to admission when moderate bleeding began. With the information available the occurrence of an abortion that had remained without overt clinical symptoms

and signs cannot be excluded definitely. The following three patients, numbers 13, 15, and 18, had histories of spotting or hemorrhage earlier in gestation. Pathological reports in these cases were not definitive, but the histories and discrepancies of uterine size might be consistent with this diagnosis, especially in the last two cases in which the duration of pregnancy would correlate well with the uterine size if abortion had actually occurred earlier. Six of these seven patients had normal or borderline BEIs ranging from 5.5 to 7.8 gamma per cent. The seventh patient had a BEI of 5.2 gamma per cent considered abnormal because seven weeks after abortion her BEI had risen to 6.1 gamma per cent.

The eighth column lists the follow up BEI values. All except those for patients 1 and 3 were obtained at least five weeks after abortion, an interval sufficient for the iodine concentrations to return to the individual's non-pregnant level. All lie within the euthyroid range except the 14.3 gamma per cent of number 1 and the elevated value of 7.1 gamma per cent of number 3. The latter's BEI was measured seven days after a Caesarean section because of a placenta previa, so that this value may be regarded as physiologically elevated by pregnancy before a return to the post partum baseline. As is indicated in Table I, follow up BEIs could not be obtained on the remaining seven women.

Thus from the 26 women who aborted (patients No. 1 and No. 4 through 28 in Table I) there are 19 cases in which BEIs at abortion and also several weeks later were obtained. For nine of these 19 patients the BEIs at abortion are essentially the same or actually lower than the values several weeks later. Eight of these nine patients are from the group with low BEIs at abortion (patients No. 19 through 28 in the table) while of the others, number 14, the hyperthyroid taking Lugol's solution, was from the group with a BEI normal for pregnancy at the time of abortion.

The behavior of the BEIs in the ten patients with low values at abortion (patients 19 through 28) and for the follow up BEIs of eight of these women is plotted in Figure 2. Between the two parallel broken lines is included the euthyroid range. Lines are shown on the graph connecting BEI values of individual patients. The symbols (identical with those in Figure 1) at the left of these lines represent on the vertical axis the abortion BEIs and on the horizontal axis the durations of pregnancy at which abortion occurred. The points at the extreme right of these connecting lines represent the BEI values after abortion, while the intervals between them signify the lapse of time after abortion. In five of the patients, numbers 20, 22, 24, 27, and 28, the rises in BEIs during pregnancy were between 0.1 and 0.9 gamma per cent. These changes are actually too slight to be regarded as significant. In three of the patients, numbers 23, 25, and 26, the

BEIs were lower in pregnancy by 0.9, 1.0, and 2.7 gamma per cent respectively. This observation of the BEI falling instead of rising in pregnancy has been noted by others.[16] Whether or not this indicates that the thyroid is primarily responsible for abortion in this latter group with low BEIs as compared with the group having normal levels during pregnancy cannot be stated.

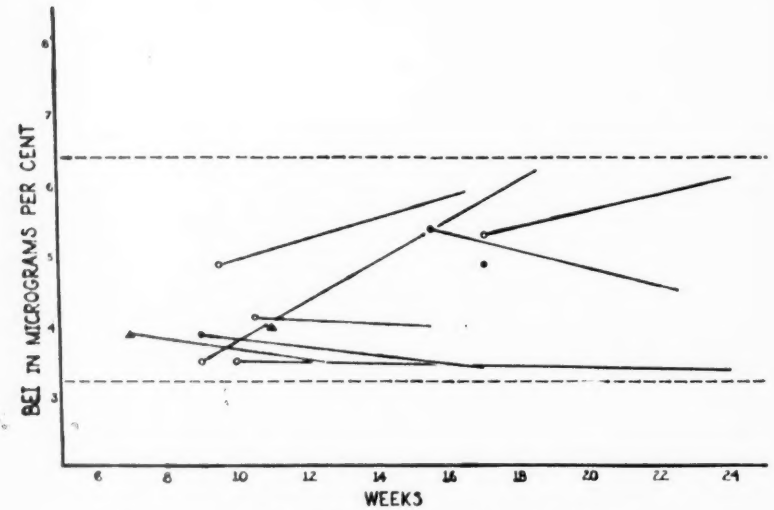

FIGURE 2. · PATIENTS WITH LOW BEIs AT ABORTION

LEGEND
- ▲ PRIMIGRAVIDA
- □ HYDATIDIFORM MOLE
- × SELF-INDUCED ABORTION
- ● MULTIGRAVIDA WITHOUT PREVIOUS ABORTION
- ○ MULTIGRAVIDA WITH PREVIOUS ABORTION
- ○? MULTIGRAVIDA WITH QUESTIONABLE PREVIOUS ABORTION

Of the group with normal and borderline BEIs at abortion (patients No. 4 through 18 in Table 1), the BEI at abortion of number 14, the treated hyperthyroid, was lower than it was nine weeks later. The BEIs of the remainder of this group ranged from 1.6 to 3.6 gamma per cent higher at abortion than the follow-up values. Approximately one third of the total number of 26 women who aborted had BEIs which did not rise or actually fell during pregnancy to levels lower than the non-pregnant baseline. This fraction might even have been higher if follow-ups could have been obtained on the patients lost to this study.

Table 2 lists the 13 patients on whom chorionic gonadotrophin values were measured at some time during pregnancy or shortly after its termination. Most of the values are of the latter category as is shown in the last column of Table 2. With these values are also quoted the BEIs which had

TABLE 2. URINARY CHORIONIC GONADOTROPHIN AND BEI VALUES IN THIRTEEN PATIENTS

Patient No.	*BEI gamma %*	*Gonadotrophin values (IU/24 hours)*	*Time of determination of gonadotrophins*
8	7.8	Greater than 132,000 Less than 388,860	10 hours after abortion
3	6.4	Greater than 18,000 Less than 45,000	5 weeks before Cesarean
14	4.8	71,000	4 hours after abortion
4	7.6	Greater than 23,000 Less than 58,800	3 hours after abortion
10	6.3	Greater than 3,000	3 days after abortion
15	5.5	Negative	? time after abortion
23	3.5	Greater than 3,000	1 week before abortion
28	3.5	Greater than 3,180	1 day before abortion
19	4.0	Greater than 6,000 Less than 12,000	10 hours after abortion
24	4.1	Greater than 12,300 Less than 37,200	7 hours after abortion
27	5.4	Greater than 30,000 Less than 50,000	6 hours after abortion
21	4.9	Greater than 1,000 Less than 3,000	4 hours after abortion
25	4.9	Greater than 1,000 Less than 3,000	20 hours after abortion

been determined simultaneously. Patient 8 represents the case with the mole and, as noted, the gonadotrophin values are extremely high. The BEI is in the range for normal pregnancy. Patient 3, the case of placenta praevia, had the expected normal values for both. The three patients, numbers 14, 4, and 10, also exhibited physiological values for both hormones. Patient 15, who had a history of three weeks of bleeding before hospitalization, had a negative gonadotrophic titer and a borderline BEI of 5.5 gamma per

cent—facts suggesting that abortion probably had occurred earlier. Five patients, numbers 23, 28, 19, 24, and 27, all had low BEIs with gonadotrophin levels greater than 3,000 IU/24 hours. There are two patients, numbers 21 and 25, in whom both values were low. Number 21 had slight spotting four days before admission, with the onset of heavy bleeding and cramping one day before admission. The other patient, number 25, had an eight-day history of spotting before the onset of heavy bleeding and the alleged passage of tissue two days before admission. Neither patient showed any evidence of thyroid dysfunction. There was no great discrepancy between uterine size and duration of pregnancy, but this does not preclude the possibility that death of the fetus and separation of the placenta occurred a few days before hospitalization. The low gonadotrophins could be attributed to this. It is certainly consistent with the histories of the patients. The explanation for the low BEIs is not apparent.

The 13 cases listed here comprise too small a group on which to base any definite conclusions about the correlation of BEI and gonadotrophic values with abortions.

DISCUSSION

Man, Peters, and associates[5, 11, 19, 25] directed attention to the critical relationships of the blood iodine levels in pregnancy. They showed that in uncomplicated pregnancy the SPI rose early, remained elevated until delivery, and returned to normal levels after a relatively short interval. The rise often reached hyperthyroid levels, being regularly above 6 gamma per cent after the first sixteen weeks in most cases but unattended by any symptoms of excessive thyroid activity. There was also suggestive evidence that failure of the SPI to reach this level by the end of four months was conducive to abortion. The data presented in this paper seem to be in essential agreement with the findings that an elevation of the serum thyroxine-like iodine in blood occurs in pregnancy and that relatively low BEI concentrations are frequently associated with abortions.

In the present series ten of the 24 patients who aborted within the first 17 weeks of pregnancy exhibited low BEIs for the duration of their respective pregnancies. This is somewhat lower than the proportion given by Man *et al.*[19] who reported that 18 of the 26 subjects who aborted in their study had SPIs lower than 6 gamma per cent. This finding of low serum iodine (either SPI or BEI) in such a large proportion of women who have aborted cannot be dismissed lightly.

All the above measurements are only on single samples of serum from blood taken close to the time of abortion and cannot be compared with

successive BEIs before, during, and after gestation. The time at which the single determination was measured in the above patients may have coincided with a period during which there was a delayed rise or temporary drop in blood iodine level as may occur in normal pregnancy as demonstrated by Man *et al.*[39] and Soule.[40] They found that in most instances the SPI or BEI rose sharply soon after the onset of pregnancy to concentrations within or near the hyperthyroid range as early as six weeks after the last menstrual period. However, in a certain proportion of the cases there was a distinct delay before the initial sharp elevation which was usually maintained. This increased level in the early as well as late stages of gestation was subject to relatively large fluctuations of 2 gamma per cent over relatively short intervals which do not occur in the stable levels of nonpregnant women. It is highly improbable that the concentration of BEI is quantitatively related to the gonadotrophic hormone since their respective increases and declines in blood levels during pregnancy differ. The BEI rises rapidly from the fifth to eighth week, continues to increase less rapidly until the tenth week when a constant level is maintained until delivery.[37] The chorionic gonadotrophin level rises in pregnancy, reaching its peak concentration between the seventh and ninth weeks, after which by the fourteenth week it falls to a relatively low level maintained throughout pregnancy.[7] From the thirteen determinations of gonadotrophin in this study, no correlation between this hormone and BEI levels is apparent. On the other hand number 1 with no adequate history of thyroid status exhibited a BEI of 15.3 gamma per cent with a gonadotrophin of less than 18,000 IU/24 hours. If the gonadotrophin value is critically low, the patient will inevitably abort. Soule[40] suggests that the rise of iodine may be due to the presence of circulating progesterone, for when Delfs and Jones[8] found low progesterone levels in abortions, there was an associated thyroid deficiency as measured by the basal metabolic rate. Comparison with this study regarding this point cannot be made since progesterone was not measured. Engstrom *et al.*[5] found that during estrogen administration to either men or women, the SPI rose sometimes to hyperthyroid levels without clinical evidence of toxicity and, with continuation of therapy, remained at a high level after the third or fourth week. After discontinuation of therapy the serum iodine gradually fell to control values. That the rise in SPI is maintained with continued estrogen administration was suggested by the elevated SPI of 10 gamma per cent of a woman who had been on stilbestrol for three months. As a result of these findings, they hypothesize that the rise of SPI in pregnancy is secondary to the early increased and continued elaboration of estrogen characteristic of normal gestation.

Man *et al.*[19] suggest the hypotheses that failure of BEI to rise in abortion may be due to a defect of the ovum or its implantation so that the stimulation for increasing iodine levels is lacking, or that a deficient response in the secretory activity of the thyroid is accompanied by a diminished or increased utilization of thyroid hormone. Hertz *et al.*[12] have shown that the thyroid uptake of radioactive iodine is increased in the pregnant rabbit, indicating a more rapid turnover of thyroid hormone. Noble and Rowlands[28] reported that pregnant women excreted less I^{131} in the first 24-hour urine specimen than non-pregnant women, a result interpreted as increased thyroid uptake in late pregnancy. Salter[31] claimed that pregnant women require and excrete unusually large quantities of iodine, an observation not confirmed by Danowski *et al.*[2] With regard to the possibility that the plasma proteins bind thyroxine and organic iodine in larger quantities than usual during pregnancy, Danowski *et al.*[2] found that the concentrations of serum albumin and globulin were lower and higher, respectively, than those of non-pregnant subjects. If serum organic iodine is bound to alpha globulin,[27] an attempt should be made to compare the iodine levels with alpha globulin concentrations during pregnancy.

Opinions have varied regarding the effect of hyperthyroidism on pregnancy. In this series only two women, numbers 1 and 14, were considered hyperthyroid. Earlier writers[1, 5, 10] suggested that spontaneous abortion may be a complication of hyperthyroidism in pregnancy, though it is generally agreed today that under proper medical treatment the hyperthyroid patient has an essentially normal gestation.[8, 11, 25] Engstrom *et al.*[7] found that the SPI tended to be more elevated in pregnant patients with active hyperthyroidism on Lugol's than in their euthyroid pregnant sisters. Since the ingestion of inorganic iodides may elevate the SPI,[3, 4] how much of the rise in these cases is due to increases of thyroxine-like iodine is not known. Actual studies of the BEIs in several hyperthyroid pregnant patients have not yet been carried out. Just as euthyroid women are able to tolerate iodine concentrations which in non-pregnant women would be indicative of hyperthyroidism,[19] the pregnant hyperthyroid is able to tolerate even higher concentrations without symptoms. Hyperthyroidism seemed to be more easily controlled in pregnancy, the requirement for antithyroid drugs decreasing during gestation and increasing after its termination. The outbursts of hyperthyroidism after delivery which occurred in some patients suggested uncontrolled activity after the effect of pregnancy. Such would seem to be the case in number 14. Just prior to pregnancy she was mildly hyperthyroid clinically, appeared euthyroid at the time of abortion and shortly thereafter, and was mildly toxic nine weeks later. There have been no reports of

SPI or BEI measurements on any significantly sized group of pregnant hyperthyroid patients receiving no medication.

In an attempt to compare BEIs during pregnancy and non-pregnancy, 19 of the 26 women who aborted had BEIs measured some weeks after abortion. As stated in the results, in nine of these 19 patients the BEI was the same or lower during pregnancy than during the subsequent non-pregnant state. All eight of the ten patients with low BEIs at abortion on whom follow-up studies were obtained are included in this group. All BEIs after abortion of the nine women are in the euthyroid range, so that there is no thyroid deficiency during non-pregnant states as measured by the serum iodine level. In the study of Man *et al.*[19] comparisons of SPIs of five women when pregnant and when not pregnant were similar to those that occur in this study. In two, the SPI fell during pregnancy. In another two, it rose in pregnancy but to levels below 6 gamma per cent, while in the remaining case it rose to 6.5 gamma per cent. In a later paper[20] they reported about eight patients with histories of miscarriages on whom both pregnant and non-pregnant SPIs were done. Half of these had non-pregnant values ranging from 4.1 to 5.6 gamma per cent which dropped during pregnancy to a range from 2.8 to 4.8 gamma per cent. The other half with non-pregnant values of 3.5 to 5.1 gamma per cent increased their iodines during pregnancy to values between 5.1 and 6.3 gamma per cent. The combined data suggest that in these cases in which there is a failure of the iodine to rise or a fall of iodine to a lower level during pregnancy, there is an improper reaction to gestation.

Earlier investigators studied hypothyroidism in pregnancy with the basal metabolic rate.[14, 15, 16, 28, 32] Using this criterion, King and Herring[14] concluded that mild or moderate hypothyroidism was a fairly common complication of pregnancy. If basal metabolic rates early in pregnancy were low, proper treatment could be started. They believed that by doing so they were able to carry many patients to term who might otherwise have aborted. In this respect Pyle[28] reported for a group of women with previous spontaneous abortions, an abortion rate of about 80 per cent prior to thyroid medication, with reduction to a rate of approximately two per cent after medication. Russell[16] found that even when the abortion was clinically inevitable, serum iodine was still frequently elevated. If, however, a low BEI were found, administration of thyroid extract might be indicated even though such therapy does not prevent abortion once symptoms have begun. Using the SPI as a criterion of hypothyroidism, Hemenann *et al.*[11] and Peters *et al.*[26] reported several cases of threatened abortions in which desiccated thyroid appeared to have been beneficial. No thyroid medication had been given to the ten women in the present series who exhibited low BEIs at abortion. It

is impossible to state that the BEI would not have increased if gestation had not been interrupted or that abortion would have been averted if sufficient thyroid extract had been administered earlier in the course of pregnancy. Parkin and Greene[24] described the occurrence of pregnancy in untreated hypothyroidism. Engstrom *et al.*[7] had several cases in which hypothyroid patients with low SPIs in spite of ingestion of desiccated thyroid during pregnancy carried to term successfully. This ingested thyroid appeared to be adequate to meet the peripheral needs of these women during pregnancy. Though in the majority of women the blood iodine lay above a certain level during gestation, there is a modicum of patients who maintain low iodine levels and who successfully complete pregnancy. Reports of the beneficial effect of thyroid and observations of the large number of low iodines associated with abortions justify the administration of thyroid medication in appropriate cases.

SUMMARY

The butanol extractable iodine (BEI) has been determined in 28 women who aborted or threatened to abort. All of the 28 were clinically euthyroid, though there were five who received some form of treatment for thyroid disorder. Of the 26 women who aborted, the BEI was found to lie below the expected range for normal pregnancy in 10. In 15 the blood iodine levels were comparable to values in uncomplicated pregnancy. In one subject the BEI was extremely elevated, indicative of hyperthyroidism complicating this pregnancy. The BEIs of the two patients who did not abort were normal for gestation.

The follow-up BEIs on 19 patients, five weeks after abortion, were all in the euthyroid range. The follow-up BEIs of nine women showed that the BEI failed to rise or actually fell during gestation. This group includes eight of the ten patients with low values at abortion. This observation suggests that the failure of iodine to rise in some patients who abort represents an abnormal response to the pregnant state.

Six patients had BEIs between 7.0 and 7.8 gamma per cent, values as high as those encountered in hyperthyroid non-pregnant women but showed no evidence of toxicity. This would seem to denote that the pregnant woman has a certain tolerance for the hormone. This would also be true of the patient who had a BEI of 15.3 gamma per cent, a concentration strongly suggestive of complicating hyperthyroidism.

The inadequate state of present-day knowledge regarding the significance of iodine rise during pregnancy and its fall or failure to rise in many abortions is commented upon. Several hypotheses in this regard are cited.

The treatment with thyroid medication of pregnant hypothyroid patients and of women threatened with abortion is recommended.

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THE METABOLIC EFFECTS OF PANCREATIC HYPERGLYCEMIC-GLYCOGENOLYTIC FACTOR (GLUCAGON)‡

HISTORY

The presence of hyperglycemia producing properties in pancreatic extracts and commercial insulin preparations was noted as early as 1923.[10, 20, 24] However, Fisher[20] believed the hyperglycemic activity resided in a "toxic fraction" which was also responsible for the local irritation and sterile abscesses associated with insulin therapy; and Collip's extracts[10] produced an unusually prolonged hyperglycemia of one to four days' duration. It is therefore uncertain whether the hyperglycemic effect of these early extracts was due to the specific hyperglycemic-glycogenolytic factor (HGF). In 1924, Kimball and Murlin[24] gave the name "glucagon" to the factor, but the earliest intensive investigators of its properties were the German workers, Burger and Kramer,[8, 11] who were dealing with the specific material and who demonstrated that the hyperglycemic effect was due to direct glycogenolytic effect on the liver.

After the first successful crystallization of insulin by Abel *et al.*[1] in 1927, Geiling and de Lawder,[16] and Burger and Kramer,[11] demonstrated that crystalline insulin, even when injected intravenously, did not cause hyperglycemia. Glucagon was therefore considered merely an unimportant impurity, and during the next few years only Burger and his colleagues in Germany continued its study. These workers were the first to attempt its purification.[9] In 1934 Scott[39] succeeded in crystallizing insulin by a new, highly efficient method which was widely adopted by most commercial manufacturers. Insulin prepared by this method was found to have hyperglycemic and hepatic glycogenolytic properties.[28] Lundsgaard *et al.* in 1939[25]

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noted that a preparation of insulin provided by Scott stimulated the release of glucose by the perfused liver, while a preparation of Abel's did not. De Duve *et al.*[24] demonstrated by means of constant intravenous glucose infusions to insulinized rabbits that Lilly insulin (prepared by Scott's method) was contaminated by the hyperglycemic factor, while a Danish preparation ("Novo") made by the Abel technique was not. Olsen and Klein[99] confirmed the hyperglycemic properties of most insulin preparations when given intravenously. "Novo" insulin was again an exception. This and other work[23, 25, 58, 93, 94, 96, 98, 100] presented strong evidence that insulin, per se, has no direct glycogenolytic effect on the liver and resulted in a shift of interest towards glucagon as an independent entity.

SITE OF ORIGIN AND DISTRIBUTION OF GLUCAGON

Sutherland and de Duve were able to extract the factor from the pancreas of several species, including man.[95, 96] They suggested that the alpha cells of the islets of Langerhans were the probable source because there were increased amounts per unit weight in the fetal calf pancreas, in the tail of the adult dog pancreas (where islets are concentrated), and in the duct-ligated and alloxan-diabetic pancreas in which acinar and beta cells have been destroyed. Pincus[78] confirmed these findings in the dog. Andy and Kerly[2] noted a similar content of glucagon in pancreatic extracts from the rabbit, rat, cat, guinea pig, and ferret and determined that in the Lophius piscatorius, a teleost fish in which islet cell tissue is found outside the pancreas, extracts from islet cell tissue had a high content of glucagon, while acinar tissue had minimal HGF activity. Sutherland and De Duve[95, 96] were also able to extract a factor, apparently identical with HGF, from the upper three-fourths of the gastric mucosa, and, in small quantities, from the duodenum and ileum of the dog, but not from other tissues. They suggested that the glucagon found in these sites may be formed by the argentophil cells of the gastro intestinal mucosa, which have a similar distribution and which stain with silver as do alpha cells. Although the two groups of cells are superficially similar, comparative morphological studies of Fodden[48] have shown that they are not related physiologically.

The site of origin of HGF has also been studied by the use of specific toxins. If glucagon is made in the alpha cells, destruction of these cells should cause a decrease in the pancreatic HGF content and an increase in the unopposed hypoglycemic action of the beta cell hormone, insulin. In 1951, Van Campenhout and Cornelis[104] reported selective destruction of alpha cells after the administration of cobaltous chloride; and Vuylsteke, Cornelis, and De Duve in 1952[105] reported a decreased HGF content of the

pancreas of animals pretreated with cobalt. Cavallero[12] reported that pancreatic extracts from fish previously treated with cobalt had no hyperglycemic effects after alkali inactivation of insulin. However, other work with cobalt has failed to confirm some of the above findings and does not support the concept of the alpha cell origin of the HGF. Goldner, Volk, and Lazarus have demonstrated that hyperglycemia following immediately after cobalt administration is not due to a predestructive "excitation initiale" of the alpha cells, as proposed by Van Campenhout and Cornelis,[13] but occurred regardless of the intervals between successive doses[10] and increased with increasing doses of cobalt[11]; indeed, the initial hyperglycemia occurred in the pancreatectomized animal.[9] The hepatic glycogen content of cobalt treated animals was decreased. They therefore ascribe the hyperglycemic response to a direct hepatic effect of cobaltous ions.[14] These authors also reported that following destruction of the alpha cells by cobalt, there was no hypoglycemia,[15] no amelioration of alloxan diabetes,[9] and a persistence of hyperglycemic activity in pancreatic extracts.[12] Raja Rama Rao and De,[24] working with rabbits, noted no decrease in HGF content of the pancreas after cobalt administration. These authors also claim to have detected HGF in the abdominal lymphatics and spleen as well as in the pancreas and gastric mucosa, and, on this basis, suggested abdominal lymphatic tissue as the source of HGF. However, they do not mention their method of extraction.

Deca methylene diguanidine hydrochloride, or Synthalin A, was shown by Davis,[25] in 1952, to produce a selective but inconstant hydropic change of the alpha cells in rabbits. These changes are not nearly as extensive as those caused by cobalt, but are followed by a hyperglycemia of several hours' duration, and then marked hypoglycemia. Fodden,[26] comparing the effects of cobalt and Synthalin A, suggested that cobalt causes a retention of HGF in the alpha cells, while Synthalin A, after causing an initial secretory discharge of HGF, inhibits completely the HGF function of the cells. He believes that this explanation reconciles the incompatabilities between alpha cell destruction and glycemic effects of the two compounds, and that his work supports the alpha cell origin of pancreatic HGF.

In summary, there is abundant evidence of the presence of a factor producing hyperglycemia and hepatic glycogenolysis in the pancreas of all species that have been assayed for the substance. The bulk of evidence supports the alpha cells as the pancreatic cellular site of origin. The main objection to this concept is the inconsistent effect obtained with the specific cytotoxins. However, for several reasons, work with the cytotoxins cannot be evaluated completely at this time. Neither of the toxic compounds has been shown to destroy all of the alpha cells, and although cobalt in some

dosages appears to produce considerable alpha cell damage, its effects on other gastro-intestinal sources of HGF are unknown. Indeed, Fodden[68] noted no effect on the gastro-intestinal argentaffin cells after either cobalt or Synthalin A. Furthermore, these substances may not affect carbohydrate metabolism entirely by damaging alpha cells, since both probably have primary hepatic glycogenolytic activity.

PROPERTIES AND POTENCY

A detailed account of the methods of extraction and the physical and chemical properties of the pancreatic HGF is not within the scope of this paper. Suffice it to say that HGF is apparently a polypeptide physically somewhat similar to insulin but differing from the latter in its amino acid composition, and its resistance to alkali and cysteine incubation.[98] It has been demonstrated that the hyperglycemia obtained with cysteine-inactivated pancreatic and gastric extracts is due to HGF and not to the hyperglycemic effect of cysteine itself.[30] HGF has been extracted in a purified crystalline form by Staub *et al.*[98] It can be assayed *in vivo* by measuring the hyperglycemic effect, but its glycogenolytic effect on rabbit liver slices[98, 109, 110] is the most accurate method. The material is extremely potent in its purified form, 0.1 μg./Kg of body weight causing a significant hyperglycemia in the cat[98]; as Foà[37] points out, on a weight basis, the potency of HGF is at least ten times greater than that of insulin.

Carbohydrate metabolism. The best-established effect of HGF is the production of a rapid, transient hyperglycemia, accompanied by marked glycogenolysis in the liver.[98] The fact that this hyperglycemia is most marked after intraportal injection,[10, 18] that it is absent in the hepatectomized animal,[92, 98] is increased when liver glycogen content is high,[59, 91] and diminished in situations where liver glycogen is decreased—as in diabetic animals in ketosis,[39, 41, 115] in patients with cirrhosis,[75] and in fasting animals[110]—all attest to the primary hepatic site of action. Sutherland and Cori[105–107] have conclusively demonstrated that the glycogenolytic effect of HGF, like that of epinephrine, is on the liver phosphorylase system, both agents probably acting upon a liver enzyme that maintains a balance between active and inactive phosphorylase. However, despite their similarities with respect to hyperglycemic and hepatic glycogenolytic effects, HGF and epinephrine have numerous points of difference. Epinephrine causes an increase in blood lactate levels, a result of its glycogenolytic effect in muscle, and this lactate secondarily replenishes hepatic glycogen stores.[21] HGF does not cause increased blood lactate levels after administration to normal animals[105, 110] or normal or diabetic human beings.[95] Furthermore, HGF does

not have the pressor influence on blood pressure and pulse that epinephrine manifests.[106]

The fact that epinephrine has effects on muscle tissue slices[20] and in the hepatectomized animal[85] is confirmatory of its peripheral, extra-hepatic activity. There are few data to support such activity on the part of HGF. The most impressive evidence of an extra-hepatic action of glucagon is the work of Drury, Wick, and Sherrill[28] who maintained eviscerated, nephrectomized rabbits at constant blood sugar levels by accurately measured intravenous glucose injections. Some were given insulin simultaneously. On the basis of the amount of glucose required to maintain constant blood levels, these authors reported that glucose transfer was decreased during HGF administration and suggested that HGF may play a rôle as an insulin antagonist. HGF, like insulin, had no effect on the rate of glucose oxidation, however. Rodriguez-Candela[97] has reported that HGF inhibits the deposition of glucose in the isolated rat diaphragm in the presence of insulin. HGF alone, however, had no effect. Young[113] was unable to confirm these results; nor could Mirsky[72] demonstrate any effect of HGF on glycogen synthesis in the rat diaphragm. Pincus and Rutman,[87] working with normal dogs, reported that HGF results in an *increase* in muscle glycogen in the presence of insulin. Engel and Scott[31] have reported that HGF had no effect on glycogen synthesis in rat adipose tissue.

Lipid metabolism. Haugaard and Stadie,[38] and Haugaard and Haugaard[37] working with rat liver slices, found a decrease in fatty acid synthesis in the presence of HGF, as measured by the incorporation of radioactive labeled acetate, glucose, or fructose into fatty acids. These authors point out that the decreased specific activity of the fatty acids produced under their experimental conditions might be explained on the basis of a dilution of the pool of fatty acid precursors with glucose released by the glycogenolysis induced by HGF but they believe that the amount of glycogenolysis was inadequate to explain the effects they observed. Haugaard and Haugaard also noted an increased ketone body synthesis in liver slices treated with HGF. Foá and Weinstein,[42,43] working with diabetic dogs, found an increased ketonemia after administration of a Lilly purified HGF, but using other crude pancreatic extracts, they noted a hyperglycemic effect with a marked *decrease* in blood ketones. These authors suggest that there is a pancreatic factor, distinct from insulin and HGF, which is capable of decreasing the blood ketone levels. Stewart and Roitman[94] obtained no effect on ketone production by rat liver slices after treatment with Lilly purified HGF or various aqueous pancreatic extracts. Zimmermann and Donovan[117] gave cysteine-inactivated insulin preparations as a source of

HGF to depancreatized dogs untreated with insulin and noted a brief increase followed by a prolonged diminution in ketonemia. These changes accompanied a similar brief hyperglycemia and prolonged hypoglycemia in the dogs. Kalant,[88] using fasted rats and intraperitoneal Lilly purified HGF, also noted a significant decrease in ketonemia acutely, and an inhibition of ketosis during fasting. It should be noted that this author reported no significant accompanying hyperglycemia nor depletion of liver glycogen, and although the absence of these phenomena can probably be explained by the fact that the animals were in the fasted state and that blood samples for analysis were apparently taken two hours after the last dose of HGF, the efficacy of HGF administered by the intraperitoneal route is open to question.[81, 89] Rutman, Pincus, Brown, and Scott,[89] found no changes in blood acetone levels of intact and pancreatectomized dogs after HGF administration. The blood lipids did not change in glucagon-treated dogs when compared to saline- or insulin-treated controls.[89] Pincus and Rutman[81] reported that HGF did ameliorate the acidosis of diabetic dogs. Bondy and Cardillo were unable to produce ketosis by intravenous glucagon in fasted normal human beings.[6]

The observed effects of HGF on fat metabolism have been contradictory. The pathways of lipid and carbohydrate metabolism are closely interrelated and converge to a common enzyme system at the level of acetyl-Co-A. The effect of glucagon on ketosis, therefore, may depend on its ability to alter the liver glycogen and the availability of glucose to the liver and peripheral tissues. The variability of the reported effects probably results from the variety of conditions under which the tests have been performed, and the consequent variations in the basic substrate on which the hormone could act. There is no clear-cut evidence that glucagon has any effect on fat metabolism except as a consequence of the primary alterations it produces in carbohydrate metabolism.

Protein metabolism. The rôle of the HGF in protein metabolism has not as yet been investigated. Tyberghein,[103] measuring urinary nitrogen excretion in rabbits, reported that HGF accelerates protein catabolism.

DIABETOGENIC ACTIVITY

The effect of pancreatectomy on experimental diabetes caused by insulin deficiency. If the pancreatic hyperglycemic factor is diabetogenic, it should be possible to alleviate established experimental diabetes caused by beta-cell destruction by removal of the remainder of the pancreas. Experiments of this type have been carried out by a number of authors. Young, in 1939,[113] reported that dogs made permanently diabetic by injections of anterior

pituitary extracts required more insulin than pancreatectomized dogs on a similar diet. Dragstedt, Allen, and Smith[25] noted that dogs who were 90% to 95% depancreatized required more insulin to maintain minimal glycosuria than a similar series of totally depancreatized animals. Cavallero and Malandra[45] reported that removal of 90% of the pancreas reduces the glycosuria and fasting blood sugar, and increased the insulin sensitivity of alloxan-diabetic rats. Thorogood and Zimmermann, in 1945,[102] reported that pancreatectomy in alloxan-diabetic dogs reduced the glycosuria and insulin requirements. These authors also noted that ketosis and coma developed more rapidly in the pancreatectomized dogs after insulin withdrawal and suggested that there might be an alpha cell hormone which acts in opposition to insulin in respect to blood sugar and glycosuria and which prevents ketosis and coma in the insulin-deficient animal.

Opposed to this explanation is the opinion of Mirsky.[78] This investigator confirmed the findings of Thorogood and Zimmermann[102] and of Rodriguez-Candela[88] that the quantity of glucose excreted in the urine by the alloxan-diabetic dog on a constant regimen of food and insulin is decreased after pancreatectomy, and that, therefore, the pancreatectomized animal will have lower insulin requirements if glycosuria is the criterion for control. However, this decreased glycosuria was not accompanied by a weight gain; after pancreatectomy of alloxan-diabetic animals, there was an increase in hyperglycemia, ketonemia, and glycosuria during periods of fasting and of insulin deprivation; despite administration of sufficient pancreatin to prevent fatty stools and fatty infiltration of the liver in the pancreatectomized dogs, such dogs absorbed considerably less protein than alloxan-diabetic dogs; the alloxan-diabetic dogs have appreciable stores of liver glycogen, since they developed a marked ketonemia only after the administration of phlorizin with consequent depletion of liver glycogen. On the basis of these findings, Mirsky concluded that the "amelioration" of alloxan diabetes by pancreatectomy is on the basis of decreased protein absorption with a resulting diminution of carbohydrate precursors, and that the resistance to ketosis and coma in the alloxan-diabetic animal before pancreatectomy is on the basis of the ability of these animals to store large quantities of glycogen. He would, therefore, ascribe the differences between alloxan diabetic and pancreatectomized animals to the exocrine rather than to the alpha cell function of the pancreas. There are certain experiments which seem to contradict this theory. Thus, Thorogood and Zimmermann[102] and Rodriguez-Candela[88] noted no decrease in the insulin requirement of the alloxan-diabetic dog after ligation of the pancreatic duct, while the removal of the remaining sclerosed pancreas did result in a decreased insulin requirement. However, Thorogood and Zimmermann reported on only two dogs subjected to duct

ligation and Rodriguez-Candela offers no details as to the observations he reported. These experiments have also been criticized because the dogs were not observed long enough after ligation of the pancreatic duct to permit a defect of protein absorption to develop.

Cavallero and Malandra[12, 13, 15] reported that pancreatic grafts from alloxan-diabetic, but not from normal, rats reduced the glucose tolerance and increased the insulin resistance of rats made diabetic by partial pancreatectomy.

Total pancreatectomy in man. In recent years a limited number of totally depancreatized human beings have been available for observation. Although many of the patients die too soon postoperatively to permit adequate metabolic study, a few have been investigated in some detail months after surgery and in a more or less stable condition. Dixon *et al.*,[28] Ricketts *et al.*,[55] and Fallis and Szilagyi[30] have reported the results of total pancreatectomy in previously diabetic patients. Summaries of studies on depancreatized humans have been published by Whipple in 1946[112] and Gourevitch, Thomas, and Whitfield in 1952.[34] In general, these patients require less insulin than many ordinary diabetics, since their maintenance dosage usually falls in the range of twenty to forty units daily. The previously diabetic patients required about the same amount of insulin as preoperatively. However, these patients all suffer from defective nitrogen and fat absorption, the number adequately studied has been small, the criteria for regulation are not uniform, and individual complicating variations are numerous, so that little clarification of the rôle of HGF as a diabetogenic agent has come, as yet, from studies of pancreatectomized humans.

Experimental production of diabetes by HGF. Cavallero and Malandra[16] reported that HGF increased the blood sugar levels and glycosuria in rats force-fed a diet high in carbohydrate, and that it enhanced significantly the hyperglycemic and glycosuria effects of pituitary growth hormone, adrenocorticotropic hormone, and cortisone. Glucagon, while not diabetogenic itself, potentiates the diabetogenic effects of cortisone in respect to glycosuria, fasting blood sugar levels, and glucose and insulin tolerance tests in intact, *ad. lib.* fed rats.[17] However, cortisone diabetes is characterized by elevated hepatic glycogen stores and the demonstration that HGF, with its marked hepatic glycogenolytic action increases the glycosuria and basal blood sugar levels of these animals is not significant evidence of a true diabetogenic effect. Ingle, Beary, and Purmalis[51] noted that HGF produced a definite but temporary enhancement of glycosuria in partially depancreatized rats. This effect was produced only when HGF was administered by intravenous

infusion, and the glycosuria returned to pretreatment levels while HGF was still being administered.

The effects of HGF have been studied in humans in an effort to determine whether a state of hyperglycemia associated with decreased peripheral utilization of glucose could be produced. Van Itallie *et al.*[105] administered HGF by infusion to normal subjects in the postabsorptive state and reported that the HGF-induced hyperglycemia was accompanied by an increased difference in the levels of arterial and venous glucose. This finding indicates that there is no inhibition of peripheral glucose transfer under the influence of HGF as there is after epinephrine administration. Indeed, the work of these authors suggested that HGF might enhance glucose transfer on the basis of comparable determinations in subjects to whom glucose had been administered. Bondy and Cardillo[8] confirmed these findings in their series, and in addition noted that glucagon and glucose caused comparable decreases in blood inorganic phosphorus and alpha amino nitrogen levels. Kirtley *et al.*[65, 66] also produced a fall in serum inorganic phosphorus levels in normal subjects during and after HGF administration. A reduction of the levels of inorganic phosphorus appears to be a reliable manifestation of peripheral hexose transfer in normal subjects.[8]

There has been no report of permanent experimental diabetes resulting from HGF administration.

Increased alpha to beta cell ratio as the etiology of diabetes mellitus. Ferner[38, 39] suggests that the anatomical basis of human diabetes mellitus is an increased ratio of alpha to beta cells, with a resulting predominance of the "glucagon system" over the insulin system. He supports his theory with morphological studies of the islets of normal and diabetic humans[38, 39, 100] as well as the aforementioned investigations relative to the effect of total pancreatectomy on alloxan diabetes, and by the well-known phenomenon of "amelioration" of pancreoprivic diabetes by hypophysectomy (with consequent removal of the alleged "alphacytotrophic principle" of the anterior pituitary). However, pancreatectomy removes the chief source of glucagon, and it is therefore most unlikely that any important part of the effect of hypophysectomy on the diabetes of pancreatectomized animals is a result of removal of the stimulus to the extra-pancreatic sources of HGF. The question of the "amelioration" of alloxan-diabetes by pancreatectomy has already been discussed. As Fabrykant[37] points out, different investigators are not in complete agreement as to the normal range of the alpha : beta ratio.[38] Furthermore, Ferner himself has demonstrated that there is an increased alpha : beta ratio in newborns, who ordinarily have low levels of blood glucose.[39]

Hyperglycemic substances in diabetic urine. One investigator[77] has reported the presence of a hyperglycemic-glycogenolytic factor in the urine of diabetic patients and alloxan-diabetic rats and rabbits, when the blood sugar levels were elevated above 200 mgm%. The action of this substance was destroyed by tryptic digestion and the author suggests that it is probably of pancreatic origin. Another report[101] of a hyperglycemic factor in diabetic urine suggests that the responsible substance is not a protein. Work in this line cannot be evaluated at this time.

HGF AS A MEDIATOR OF THE EFFECTS OF GROWTH HORMONE

It has been suggested that there is a relationship between an anterior pituitary "alphacytotrophic factor" closely related to growth hormone (STH) and the HGF, and that the HGF may mediate the diabetogenic effects of STH.[12,85] The effects of purified growth hormone are reviewed by Young.[104] For the purposes of this discussion it can be pointed out that in animals these preparations have well established growth accelerating properties which can be measured by epiphyseal cartilage growth or by the production of positive nitrogen balance, and that they have definite diabetogenic properties in some species. The evidence linking the growth hormone and HGF is as follows:

Histologic studies after hypophysectomy or administration of anterior pituitary extracts. Somenberg[92] reported that isotopically-labeled growth hormone is concentrated in the pancreas. Ferner[36] found that hypophysectomy was associated with atrophy and a decrease in number of alpha cells, with no alterations in beta cells in all of the guinea pigs, and in one-half of the rats in his series, but in five or six months the cell pattern returned to normal. He also reported increased numbers of alpha cells as compared to the number of beta cells in human embryos and newborns and cited this finding as evidence associating the growth hormone, alphacytotrophic factor, and alpha cell system. Ham and Haist[55] treated dogs with daily injections of anterior pituitary extract and reported evidence of alpha cell degranulation in a small percentage of their animals. Cavallero[12] reported that rats pretreated with growth hormone preparations showed a marked increase in the ratio of mitotic activity of alpha cells to beta cells. Similar observations were made in dogs made diabetic by anterior pituitary extract injection. They also treated normal dogs with pancreatic extracts from these "Young diabetic" dogs and from normal animals and reported a more marked and prolonged hyperglycemia in the former animals.

Secretion of HGF in response to growth hormone. Bornstein, Reid, and Young[7] noted a hyperglycemic and glycogenolytic effect of portal or

pancreatico-duodenal venous blood but not of peripheral blood in intact and adrenalectomized hypophysectomized alloxan-diabetic animals treated with growth hormone. Growth hormone by direct injection and blood from saline treated control animals failed to elicit the HGF effects in the animals used for assay. Foà *et al.*[40] reported that blood from the pancreatico-duodenal vein, but not from the mesenteric vein of donor dogs treated with growth hormone, caused a transient hyperglycemia in recipient dogs that was similar to the typical response to HGF. Blood from control animals injected with control solutions of the identical pH had no hyperglycemic effect.

It is important that growth hormone is capable of producing permanent diabetes in experimental animals,[34] but that HGF, even in high sustained dosages, is not. This does not support the concept that the diabetogenic effects of growth hormone are mediated entirely by HGF.

Somatotrophic effects of HGF. Edrick[37] treated hypophysectomized rats with HGF and reported that in most instances there was a significant increase in epiphyseal cartilage growth as compared to controls. He suggested that both the diabetogenic and growth stimulating actions of growth hormone are mediated, in part or wholly, through the release of HGF. However, Geschwind and Staub,[38] using highly purified HGF preparations, were unable to confirm these results.

CURRENT STATUS

Is glucagon a hormone? It is a substance which exerts its effects on a distant organ and is potent in minute doses. It probably is formed chiefly in the alpha cells of the pancreas. Cavallero points out[17] that these cells have the characteristics of an independent endocrine organ, since they show morphological evidence of secretory activity, and their reactions to cytotoxins and physiological and pathological conditions are independent of the beta cells and the acini. The cross circulation experiments of Foà and his collaborators[42, 46] have established the fact that the pancreas secretes a hyperglycemic substance into the blood stream in response to insulin induced hypoglycemia. Work with perfusion of the isolated rat pancreas suggests the same conclusion.[7] It has been impossible, however, to produce a pure and total deficiency of HGF. The rapid action and potency of glucagon and the evidence afforded by cross circulation experiments indicate that the HGF is probably one of the factors responsible for the regulation of blood glucose levels in the intact animal, possibly in response to anterior pituitary stimulation under some circumstances. Engel suggests[20] that HGF, like epinephrine, is involved in the early response to hypoglycemia. However,

the failure to obtain permanent hypoglycemia after destruction of most, if not all, of the alpha cells by cobalt, the demonstration that the response to hypoglycemia is not altered by pancreatectomy,[26, 36] the fact that liver glycogen is not diminished in alloxan-diabetic animals[23, 38] and the transient nature of HGF effects make it unlikely that the HGF plays a dominant rôle in metabolic homeostasis.

Glucagon is often referred to as an "anti-insulin" factor. This statement is true only in the most limited sense. It is true that, whereas insulin reduces the concentration of glucose in the blood, glucagon raises it; but these effects are produced in totally different sites and by unrelated mechanisms. Thus insulin appears to act chiefly on the peripheral tissues by increasing the entry of glucose into the cellular metabolism,[28, 29] whereas glucagon acts chiefly by promoting the breakdown of liver glycogen and its release as glucose into the blood. It is therefore clear that glucagon and insulin act synergistically, the former by making liver glycogen stores available for the peripheral action of the latter. In insulin-deficient animals[17, 30, 40, 41] and human beings before hepatic glycogen is depleted[30] the hyperglycemic effects of HGF are exaggerated, but this is doubtless because under these circumstances the second link in the chain—insulin—is absent.

There is, therefore, no convincing evidence of any peripheral effect of HGF; indeed, at this time, the only effects that can be considered established are its hyperglycemic and hepatic glycogenolytic properties, which were demonstrated by Bürger and Kramer more than thirty years ago.

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PAPER ELECTROPHORETIC STUDY OF A RAT SUBMAXILLARY GLAND EXTRACT†

A number of physico-chemical studies on the secretory products of salivary glands have been reported, but only a few scattered data appear available concerning constituents of the total salivary gland. In regard to electrophoresis, very few studies exist pertaining to any salivary gland protein components. Phase boundary electrophoresis studies on mucin extracts from animal salivary glands have been reported by Blix,[1] McCrea,[10] and Caspary.[2] In this study a paper electrophoretic technique was used to study some soluble protein components of extracts from whole submaxillary glands of rats.

METHOD

Young adult, Wistar strain, male rats were used in all of the experiments. Body weights ranged from 200 to 300 grams. Each rat was anesthetized with ether, and the jugular veins exposed. One vein was severed and, after considerable blood loss, 40-50 ml 0.9% NaCl was infused intravenously in order to wash out as much blood as possible. Then the two submaxillary glands were removed and immediately placed in a mortar full of small dry ice chips. As soon as the tissue was frozen, sand was added and the mixture was ground with a pestle to a fine powder. At the close of comminution in a cold room (4° C.), the dry ice vaporized; then the powdered tissue-sand mixture was transferred to a centrifuge tube, and 2-4 ml. phosphate buffer (pH 6.9, ionic strength 0.1) was added. (Of several buffers tried, phosphate gave the best results.) The mixture was stirred for ten minutes, then placed in a centrifuge with a pin type head for seven minutes at 3,000 rpm. The supernatant fluid was decanted off and either used for electrophoresis immediately or stored in the cold room and used within 48 hours.

Paper electrophoresis was performed with the apparatus of Kouw *et al.*[8] Acetate, phosphate, and veronal buffers (pH 4.5-8.6, ionic strength 0.01-0.1) were used along with Munktell No. 20 filter paper. The potential was 100 V, currents ranged from 1.9-2.5 mA per strip, and running time was 12 hours. Amount of sample applied was 0.1 ml.

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After completion of electrophoresis, the strips were placed in a 70° C. oven to dry for half an hour and subsequently were stained with amido-black.[5,6] After staining, a photometric device*[7] was used to scan and measure the intensity of the stain along the patterns.

EXPERIMENTAL

In one experiment, seven animals were sacrificed and the submaxillary glands removed and treated as above, in order to test the reproducibility of the method of preparation.

In another experiment, six animals were starved for 12 hours, then 30 mg. of L-methionine containing about 300 μc. sulfur[35] and dissolved in 0.5 ml. physiological saline was injected intraperitoneally. Seven hours later the animals were sacrificed and the submaxillary glands were removed and treated as above. After electrophoresis, the stained filter papers were scanned with the photometer, then each paper was cut across into 4 or 5 mm. strips. These small strips were placed all with the same side up in aluminum planchettes and the radioactivity was measured in a windowless, preflush flowchamber (Tracerlab) with a counting accuracy of better than $\pm 2\%$.

In another experiment, three rats were exposed to whole body x irradiation before injection of S^{35} labeled methionine. X irradiation was carried out with use of a 200 KV x ray machine at 75 mA.‡ One rat received 100 r four days before injection, the second rat 200 r 20 hours before, and the third rat 300 r four days before. The submaxillary glands were removed and treated as above. After electrophoresis, measurements were made on the filter papers in the same way as before, first with the photometer, then with the flow chamber.

RESULTS

Of the various buffers tried, the use of veronal resulted in the clearest separation of the main fractions. Photometric measurement of the electrophoretic patterns were reported as curves as in the example in Figure 1. Four separate components can be noted, all migrating toward the anode. In all experiments these fractions appeared consistently. The fastest moving fraction "A" was always distinct from the second fastest moving fraction "B." The third fraction "C" and the slowest moving fraction "D" were

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‡ For x irradiation the author wishes to thank Dr. C. H. Chang, Department of Radiology, Yale University School of Medicine

less clearly separated and it appeared that each might contain several subfractions. Fraction D sometimes seemed to separate into two components.

The percentage distribution of the proteins of the four main fractions was estimated. The fractions were parted by dropping a perpendicular from the lowest point between two adjacent peaks to the abscissa. A planimeter was used to measure the areas under each peak. Results on 13 rats are shown in Table I. Fraction D was consistently greater than Fraction C, but Fraction B always appeared less than Fraction C. Fractions B and A relatedly varied; either they were about equal or B was greater.

In some instances a heavy precipitate stayed at the starting point, i.e., the place where the sample was applied. This mainly occurred when cen-

TABLE I. PERCENTAGE DISTRIBUTION OF THE EXTRACTED SALIVARY GLAND PROTEINS DETERMINED IN 13 RATS

A	*B*	*C*	*D*
13.8* ± 1.05†	15.6 ± 11.9	26.9 ± 0.87	43.7 ± 1.84
27.4‡	27.5	11.7	15.2

* Mean.
† Standard error of mean.
‡ Standard deviation as percentage of mean.

trifuging failed to clear the sample sufficiently. After electrophoresis the glass plate-filter paper-glass plate "sandwich" was disassembled by removing either the top or bottom glass plate. The filter paper-glass plate remainder was dried horizontally with the paper up. If a precipitate was present on the side of the paper where the sample had been applied and this side was down during drying, after staining one could observe on the other (up) side the soluble proteins which stayed at the starting point. The precipitate masked photometric measurement of these soluble proteins, but the effect was visible to the naked eye.

Results with rats previously injected with S^{35} labeled methionine may be seen in Figure 2. The location of the radioactivity fairly well coincided with the location of peaks of stain intensity. In Fraction A there appears a slight difference in location; the stain was ahead of the radioactivity. Several subfractions in C and D, indicated by the staining, also appear in the measurements of radioactivity.

A few preliminary experiments showed that x-irradiation caused no changes in the electrophoretic pattern of protein components of rat sub-

maxillary glands as assessed by amido-black staining. The relative amounts of soluble protein fractions were quite similar in the various samples obtained from animals receiving different amounts of radiation. However, the irradiation did seem to affect the bio-labeling of the proteins following the injection of S^{35}-labeled methionine. After 100 r, the distribution of radioactivity in the different protein fractions appears the same as in the control samples. However, after 200 r, Fraction A displayed a considerably higher amount of radioactivity than that of the control. After 300 r, an

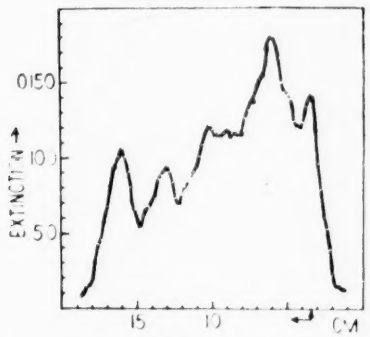

FIG. 1.

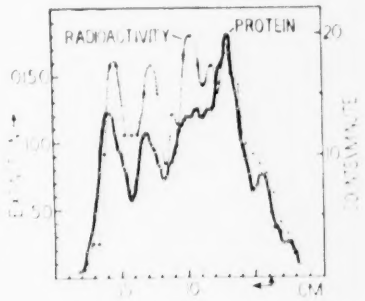

FIG. 2.

FIG. 1. Graph and photograph of an electrophoretic pattern of a rat submaxillary gland extract. The arrows represent the starting point and the direction of migration toward the anode. The curve is the photometric measurement of the intensity of the amido black staining.

FIG. 2. Protein and radioactivity distribution along an electrophoretic pattern of a submaxillary gland extract from a rat previously injected with S^{35}-labeled methionine. The arrows represent the starting point and the direction of migration toward the anode. Curve labeled "protein" is the photometric measurement of the intensity of the amido-black staining. Other curve is radioactivity measured from strips of the same pattern.

additional effect was noted; one large, single confluent peak could be seen corresponding to the location of Fractions B and C.

DISCUSSION

Demling, Kinzlmeier, and Hemming[1] reported results of an electrophoretic study of various tissue extracts, e.g., lymph node, liver, skin, etc. These authors emphasized the many technical difficulties one has to overcome in obtaining an extract containing native tissue proteins without considerable denaturation. In our case, mere grinding of the tissue with sand yielded unsatisfactory results. The extract contained insufficient amounts of

proteins for facile electrophoresis, and, in addition, the faint, diffuse fractionation which resulted was interpreted as a sign of considerable denaturation of the proteins. Our technique of freezing the salivary gland immediately after removal, and subsequently keeping the tissue at low temperatures during the preparative procedure, provided a convenient concentration of proteins for electrophoresis and, as far as could be judged from the sharp, distinct fractions on the patterns, denaturation was largely prevented.

The electrophoretic results reported in this paper represent those cytoplasmic proteins which are soluble at pH 6.9 in KH_2PO_4, Na_2HPO_4 buffer. We found that the use of this eluant produced the best electrophoretic patterns. (One should bear in mind that composition of a tissue extract is largely determined by the dissimilar solubilities of the different proteins in it. In this study it is realized that the electrophoretic patterns consisted of only those salivary gland proteins which were soluble in the eluant used.) It appears quite possible to be able to obtain different results with other eluants.

One can assume that our results represent true tissue proteins with insignificant contamination from nonspecific intercellular plasma proteins. If our electrophoretic patterns contained large amounts of plasma proteins, one would expect to find a high peak at the place of albumin but this was never observed.

The S^{35} labeled methionine became incorporated into all of the protein fractions of the salivary gland extract. Although the number of these experiments was small and they should be considered qualitative and preliminary in nature, some quantitative reasoning appears possible. The radioactivity per unit amount of protein appears to be considerably less than that of the plasma proteins. The rate of turnover of the plasma proteins, as determined with S^{35} labeled methionine, is fairly well established,[11, 12] but there are no similar data available pertaining to the turnover rate of the salivary gland proteins. The considerably smaller degree of biolabeling of the salivary gland proteins, as compared to that of the plasma proteins in the same animal, could be attributed to at least three things: (a) lower methionine content of the extracted proteins, (b) slower turnover rate, (c) very rapid rate of synthesis. This latter event may be so rapid that radioactivity of the protein extract is already low seven hours after the methionine injection; also this latter interpretation appears to be the most plausible, particularly since the salivary gland produces and excretes a considerable amount of protein. This, added to the general cytoplasmic turnover, may result in a rapid elimination of radioactive proteins from the gland. Further studies, varying the time between the methionine injection and the removal of the gland, are needed to solve the problem. It

is felt that the use of labeled amino acids offers an effective way to study the dynamic processes of the production of the salivary gland proteins.

In paper electrophoretic studies of the plasma proteins, with use of S^{35}-labeled methionine, the position of peaks of stain intensity and the peaks of radioactivity were in identical locations.[4] In our study, there is a dislocation in the peaks of Fraction A, as assessed by staining and compared with the measurements of radioactivity. This discrepancy does not seem attributable to the technique; similar methods produced agreement with plasma proteins. Two possibilities are proffered in explanation: (i) a minute amount of plasma proteins present in the gland extract could cause a peak near Fraction A, the location being near to that of albumin. Taking into consideration the much higher specific activity of the plasma proteins, a contamination appears conceivable. (ii) Also there may be a salivary gland protein migrating at this rate which has a greater specific activity than the other proteins migrating to or near the same spot. Further studies, adding known amounts of radioactive albumin to the gland extract, are being planned to clarify the picture.

The results obtained following whole body x irradiation also should be considered qualitative and preliminary. The experiments were designed to test the responsiveness of the salivary gland to ionizing radiation; therefore, medium dosages were used. The changes observed indicate considerable radiosensitivity of this gland. In addition, the methods seem to offer opportunity to determine the minimal dosage of x-rays which will interfere with the protein turnover of the gland. Such information would be valuable, particularly in regard to the known consequences of therapeutic radiation delivered in the neck region which often are followed by rapid decay of the teeth in humans. Another interesting correlation, one might point out, concerns postirradiation infection originating in the mouth as a possible result of alterations in the salivary constituents. These problems seem suggestive for further follow-up.

SUMMARY

A method was developed to extract salivary proteins with minimal denaturation.

Paper electrophoresis studies consistently demonstrated four distinct fractions.

Intraperitoneally injected S^{35}-labeled methionine became incorporated into all fractions. When tested seven hours later, the radioactivity of all salivary gland proteins extracted was significantly lower than the radioactive content of the serum proteins.

X-irradiation (200-400 r) grossly changed the biolabeling of the salivary gland proteins.

Further use and potentialities of this new method were discussed. The possible clinical significance of the radiosensitivity of the salivary gland was pointed out.

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AN ANTIBACTERIAL EFFECT OF SALIVA DEMONSTRATED WITH USE OF PAPER ELECTROPHORESIS‡

Various antibacterial factors in saliva have been demonstrated,[1,2,12,13] but none seem to have been isolated. A "lytic" agent in saliva and a purified lysozyme from eggwhite seem similar in their effect on *Micrococcus lysodeikticus*,[5,9,11] although the two have been shown to be immunologically distinct.[10] Inasmuch as separation of protein elements in saliva, by means of paper electrophoresis, has been reported,[8] it appeared possible to determine if any of the salivary protein fractions displayed an antibacterial effect. In this note, after electrophoresis of whole human saliva, the filter paper strips were placed on top of cultures of *M. lysodeikticus* to observe whether or not the antibacterial effect would correspond to the location of any protein elements in saliva.

METHOD

Saliva samples were collected on ice from six healthy subjects. Samples were centrifuged immediately, and the supernatant fluid was lyophilized by vacuum freeze drying. The residue was re-dissolved in enough phosphate buffer to effect a ten times concentration. (The residue was also re-dissolved in other buffers, but the best results were obtained with phosphate buffer.) Some concentrates were applied directly to the filter paper; or if it seemed necessary, due to cloudiness of the sample, others were centrifuged and the supernatant fluid applied. The amount used was 0.1 ml. in both cases.

Paper electrophoresis was performed with the apparatus of Kouw, *et al.*[7] Acetate, phosphate, and veronal buffers, pH 4.5-8.6, ionic strength 0.025, were used along with Munktell *No. 20* filter paper. The potential was either 100 or 125 V., currents ranged from 0.1-1.5 mA per strip, and running time was 12 hours. After electrophoresis, the wet strips were cut longitudinally in halves and one half was dried and stained with

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amido-black[3,4] for protein, while the other half was used immediately in the procedure cited below.

The test organism, *M. lysodeikticus* (ATTC 4698), was transferred from an agar slant into peptone broth. Fifteen ml. of fresh nutrient agar (23 gm. agar and 8 gm. NaCl per liter distilled water) was pipetted into test tubes kept in a water bath at 45° C., and 0.5 ml. of the culture suspension was added. This mixture was poured into Petri dishes of various sizes which were shaken gently to distribute the culture evenly into the medium. After the mixture had jelled, the wet, unstained half of the filter paper strip (cf. above) was placed on top of the agar in the seeded plate. Plates were incubated at 37° C. for 24-48 hours.

RESULTS

After incubation, the stained half of the paper strip was realigned by means of previously marked identification lines on top of the half in the Petri dish. The examples in Figures 1 and 2 show the stained protein location and the areas of inhibited growth at two different pH's. In all of the 44 electrophoretic runs, the effect of saliva on the bacteria could be noted, extending from the place of sample application toward the cathode. The effect, observable about one cm. each side of the starting point, may be attributed to initial diffusion of the saliva sample at the time of application and some irreversible absorption to the filter paper (cf. below, saliva passed through filter paper). A strong absorption of the lytic factor to the paper during electrophoresis also is evidenced by the "trailing" toward the cathode. This absorption masks any antibacterial effect which may result from fractions of slower migration rates. One may conclude that there is an antibacterial fraction in saliva which migrates similarly to the faster of two separated protein fractions in saliva at pH 6.9 and which migrates similarly to the main salivary protein fraction at pH 4.5.

When saliva is passed through a filter, it loses its lytic effect.[2] This may be demonstrated by soaking filter papers with fresh or concentrated saliva and placing them on top of the agar in a Petri dish seeded with *M. lysodeikticus*. After 24 hours of incubation at 37° C., zones of inhibition could be seen around the periphery of the paper disc. Similar samples soaked with saliva which had been filtered (with use of glass wool, various weights of filter paper, Seitz filters, or membrane ultrafilters) showed no zones of inhibition. Examples may be seen in Figure 3.

By means of paper electrophoresis, it appears possible to demonstrate a relation between the cathodic migration of separated salivary protein fractions and an antibacterial effect on *M. lysodeikticus*. This antibacterial effect was demonstrated with saliva from healthy persons; opportunity is offered to determine if any differences exist in saliva from persons with pathological states. Further investigations are in progress.

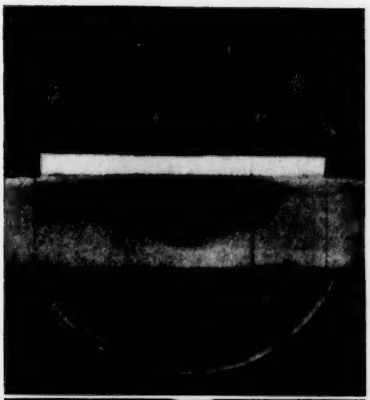

FIG. 1. Protein distribution and antibacterial activity along an electrophoretic pattern of 10 times concentrated human saliva. Line with arrow represents the starting point. Arrow points toward the anode. Electrophoresis strip was cut longitudinally in halves. In the picture the stained half is superimposed on top of the other half which has been incubated in a Petri dish seeded with *M. lysodeikticus*. Run was 12 hours, 100 V, 0.8 mA, phosphate buffer, pH 6.9, μ 0.025, Munktell 20 filter paper.

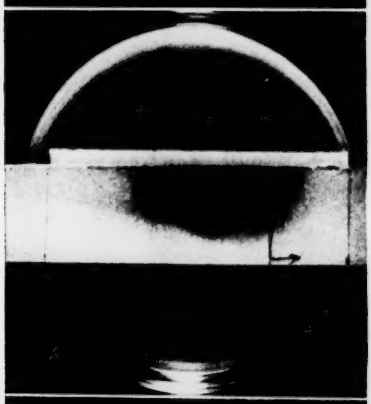

FIG. 2. Protein distribution and antibacterial activity along an electrophoretic pattern of 10 times concentrated human saliva. Line with arrow represents the starting point. Arrow points toward the anode. Electrophoresis strip was cut longitudinally in halves. In the picture the stained half is superimposed on top of the other half which has been incubated in a Petri dish seeded with *M. lysodeikticus*. Run was 12 hours, 125 V, 0.8 mA, acetate buffer, pH 4.5, μ 0.025, Munktell 20 filter paper.

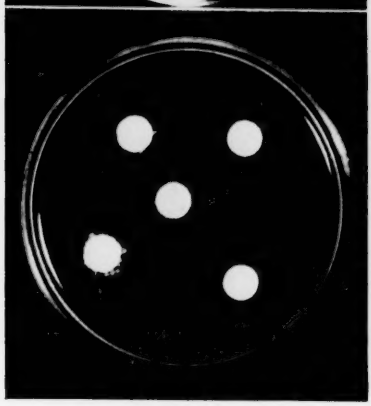

FIG. 3. Antibacterial activity of filtered and unfiltered human saliva. Paper discs impregnated with saliva were placed in a plate seeded with *M. lysodeikticus*, then incubated. Right-hand discs were soaked with unfiltered saliva. Left-hand discs were soaked with saliva which had been passed through filter paper. Center disc was soaked with sterile water.

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EVENTS

YALE MEDICAL SOCIETY

October 10, 1955

THE SURGICAL PHYSIOLOGY OF PORTAL HYPERTENSION. By Charles Gardner Childs, III, Professor of Surgery, Tufts Medical College, Boston.

Recent work, in which dye is injected into the superior mesenteric vein, thus outlining the portal and tributary circulation, has revealed no congenital anomalies in the 250 patients studied, though rare anomalies have been described where the portal vein empties directly into the inferior vena cava or into a pulmonary vein. Portal venograms applied to patients with carcinoma of the head of the pancreas have outlined the many collateral vessels which bypass the portal block; chief among these is the inferior mesenteric vein.

The portal circulation of the monkey, as in man, is retroperitoneal, whereas in the dog it is mesenteric. In studies of the effects of portal block on collateral circulation the *Macaca mulatta* monkey was used. Following sudden ligation of the portal vein, there is (1) a sharp, acute rise in portal pressure which returns to normal in six to ten days following (2) the development of such large collateral channels as the inferior mesenteric, retropancreatic, duodenal, right colic, coronary, and splenic veins, and (3) there is an immediate drop in femoral artery pressure which returns to normal within two hours. Identical portal block was performed, during the course of operation, in 25 patients with far advanced abdominal tumors. As in the monkey, the acute portal hypertension was well tolerated. Hence it is evident that "portal circulation has infinite capacity to bypass surgical occlusion." An operation for carcinoma of the pancreas has been devised where the head of the pancreas, duodenum, and portal vein are resected. It is too early to evaluate this procedure in the eight patients done to date.

In patients with portal hypertension and bleeding varices secondary to cirrhosis, where surgery is indicated, portal venograms demonstrate the patency and caliber of the portal and splenic veins, and are of great value in choosing between spleno-renal and portal-caval types of shunt. Portal-caval shunt is the procedure of choice because of the large size of the vessels involved. There is an average drop in pressure of 20 cm. saline following portal-caval shunt; these shunts, contrary to prevalent opinion, do not obliterate if properly performed. Of 56 patients having portal decompression, only one bled again. However, there is a 14 per cent postoperative mortality rate. Therefore, in contemplating portal decompression one must weigh the likelihood of further bleeding against the significant mortality. Patients with cirrhosis following hepatitis benefit most from portal decompression. Since it is liver failure which often accounts for the postoperative mortality, decompression is less beneficial in advanced alcoholic cirrhosis.

J. C. C.

ZOOLOGICAL JOURNAL CLUB

October 19, 1955

THE DISTRIBUTION OF T-ALLELES (LETHALS) IN POPULATIONS OF WILD HOUSE MICE (MUS MUSCULUS). By L. C. DUNN, Professor of Zoology and Director of the Institute for the Study of Human Variation, Columbia University, New York City.

The work described is still in progress, so the seminar was in the nature of a progress report. The genes being studied are a series of "genetical alleles" (not necessarily point mutations at one locus) clustered in a small region of chromosome IX in the house mouse. The series as a whole begins to exert its influence during early stages of embryogenesis, different members beginning at different stages (some even before implantation of the zygote). The biochemical mechanisms involved are unknown, but many morphological features are influenced, marked tail shortening being the most obvious and the one studied. No modifying factors have been found which completely mask this influence. The members of the series are designated + (normal), T, t^0, . . . , t^{12} (for laboratory populations), and t^{w1}, . . . , t^{w14} (for wild populations).

Genotypes with their associated phenotypes are: +/+ = normal; T/+ = brachy tail (about ½ normal length); T/t (t being either t^n or t^{wn}) = tailless; T/T = lethal (dies after about eleven days of development — lacks notochord and other axial structures); t^n/t^n or t^{wn}/t^{wn} = almost always lethal (various defects); t^a/t^b or t^{wa}/t^{wb} = generally lethal, but certain combinations viable and normal. At least some of the "genes" of the t^n series are probably actually chromosomal modifications, e.g., t^0 is a crossover suppressor for 5.6 crossover units on both sides of itself.

These genes were first noted in laboratory populations. At least twelve such have been identified so far. Just how they arose is unknown. An estimate of laboratory mutation rate which gave extremely odd results (7 mutations in 3,500 mice studied!) led to the present study of wild populations.

Detection of one of these genes in a wild mouse involves analysis of offspring following mating with a known T/+ (brachy). Any tailless offspring show the presence of a gene of this series. Crossing these tailless mice (T/t^{wa}) with other tailless mice of known genetic constitution gives some idea of whether the gene found is new, or merely one of those already known. Such crosses also can give evidence of further changes in already known genes (one possible case of this found so far).

Of 18 wild populations so far tested, 13 had at least one of these alleles (how many of these are different from one another and also from the laboratory genes is still uncertain). The populations studied are from all over the United States, with one from Japan. Marked geographical differences in occurrence are indicated, e.g., with only one exception so far, populations from northern New England do not have these genes. The frequencies of these genes in wild populations in which they occur are very

high, approximately 50% of such populations being heterozygotes carrying one of them. No tailless phenotypes have been found in the wild yet, however.

The evolutionary significance of, and the mechanism for, maintenance of these genes in wild populations are two problems of great general interest being studied. Experiments designed to estimate any selective advantages possessed by heterozygotes over homozygous normals are in progress. Similar studies of the maintenance mechanisms are also in progress. Factors being studied are mutation rate, relative numbers of gamete types produced (in cases studied so far t-carrying gametes are by far the most numerous), and fertilities of different genotypes. Various associated embryological and physiological problems are also being investigated.

MALCOLM S. GORDON

PSYCHIATRY RESEARCH SEMINAR

October 21, 1955

PHARMACOLOGICAL APPROACH TO PSYCHIATRY. By JOEL ELKES, Department of Experimental Psychiatry, Birmingham University Medical School, England.

The author presents a point of view aimed at elucidating the use of drugs in the treatment of psychiatric disorders rather than extensive experimental data from various fields of investigation. In such investigations three major sources of information should be utilized, namely, (i) physiological mechanisms and the pharmacological effects of the drugs, (ii) effects on the behavior of animals, and (iii) effects on patients in the controlled clinical trial.

This last aspect, most significant from the clinical point of view, is exemplified by an experiment with chlorpromazine on 27 psychotic patients. It is imperative that the trial be carried out over a long period of time, preferably a year, to eliminate the extraneous effects which may be encountered in such an experiment. The blind use of placebo is essential in evaluating the effects of the program on the hospital staff and other persons involved, since enthusiasm runs high initially in these trials and is reflected in the behavior of the patients. After four to six weeks this enthusiasm wanes, and the effects of the drug can be judged. The results must be confirmed by the alternate use of the drug and placebo many times on the same patient to rule out fortuitous improvement not related to the properties of the drug. Such was the case in this trial. Seven of the 27 patients showed definite improvement on chlorpromazine after the initial six weeks had passed. They relapsed on placebo and improved time and again when placed back on the drug. This trial was carried out for almost a year and leaves little room to doubt that this drug effects an improvement in some psychotic patients.

L. Z. B.

PHI DELTA EPSILON ANNUAL LECTURE

October 24, 1955

THE PATHOPHYSIOLOGY AND MANAGEMENT OF HEADACHE. By H. Houston Merritt, Professor of Neurology, Columbia University, New York City.

"Headache is the most common symptom mankind is subject to" and may be a manifestation of serious disease, or just minor physiological disturbance. Numerous diseases involving any organ system may be incriminated, though in the majority of headaches etiology is unknown.

The pain of headache is mediated by the Vth, IXth, and Xth cranial nerves and cervical nerves and is thought to be caused by (1) traction on the veins, arteries, and dura at the base of the brain, (2) changes in caliber of cranial vessels, and (3) inflammation around these structures. The nature of the pain is often indicative of the pathology involved, e.g., migraine headache is usually unilateral, that of hypertension pulsating, that due to intracranial tumors is boring and consistently on the side of the lesion, while in "tension" headache the patient complains of a tight band about the head.

Excluding trauma, hypertension, and intracranial neoplasia, headaches can be divided in two major groups, migraine and "tension." Two per cent of all persons suffer from migraine attacks, females four to five times more often than males. While migraine headaches are variable and may simulate any of a large number of conditions, in the typical attack the onset of the throbbing, unilateral pain is often preceded by prodromata consisting of focal neurological symptoms, usually ophthalmic in nature, such as scotomata, flashes of light, or temporary blindness; often an attack is heralded by tingling of the opposite arm or leg. Following the onset of pain, gastrointestinal symptoms such as nausea and vomiting are not uncommon. The attacks may last from 30 minutes to 72 hours, with an average of six to eight hours.

Inasmuch as the headache of migraine is known to be related to changes in caliber of the cranial vessels, vasoconstrictive agents aid in the control of the attacks. Dihydro ergotamine tartrate, alone, or combined with caffeine, if given within one hour of the onset of the attack, is extremely effective. Surgical ligation of the superior temporal and/or middle meningeal arteries has been of benefit in some cases. Long term management consists of psychotherapy and elimination of the emotional stress which often triggers an attack.

Tension headache occurs equally in both sexes and is most common in early middle life. The pain is of a chronic, persistent nature and the patient often complains of a tight band about the head. While there may be some correlation with changes in caliber of cranial vessels, this type of headache is best related to excessive tension in the neck muscles, as shown by electromyographic studies. Treatment consists of analgesics and sedatives during the acute attack and psychotherapy between attacks.

J. C. C.

BOTANY-ZOOLOGY SEMINAR

November 9, 1955

MALTHUS AND THE MODERN WORLD. Karl Sax, Professor of Botany, Harvard University.

The fact that man's biotic potential, as that of virtually all other organisms, if unchecked is great enough very rapidly to inundate the earth in a sea of life was recognized by Thomas Malthus in the early nineteenth century. Malthus also recognized that, imperfectly controlled as it is, this great potential is causing a rate of population increase far in excess of man's ability to expand his food and energy resources. The same situation exists today, in far more acute form.

Malthus recognized two types of checks for at least slowing the process: positive (e.g., a high death rate) and preventive (e.g., a low birth rate). He had little faith in preventive checks, hence concluded that poverty is inevitable and justified. Such a position is hardly justifiable today, hence preventive checks must be invoked. People who feel that things will level out of themselves some time in the future need only consider present conditions and their trends for it to become apparent that positive steps must be taken.

The human population of the earth is growing at the rate of more than 1.2% per year. Many nations are increasing their populations at rates greater than 3% per year. This high rate, unprecedented in human history (up an estimated ten times over the rate during the pre-Christian era, up some four times over the rate 300 years ago), if continued will result in a world population of some four billion by the year 2000.

This increased rate is correlated with the greater part of the world's population being in the intermediary stages of what may be termed a "demographic transition" (a change from high birth and death rates with low living standards to low birth and death rates with high standards). Lowered death rates resulting from the application of the most rudimentary public health measures are to a great extent the cause of the present increase in rate of growth. A combination of circumstances, perhaps most notably the discovery of the New World, allowed the nations of the Western world to complete this transition in great part. Unfortunately, the requisite lands and resources for completing the transition seem not to be available in much of the rest of the world.

Most of the world is now underfed. In Asia the total land available is at present less than one acre per person. Doubling agricultural production might just feed the present population adequately—it would not leave anything over for the expansion and changes associated with the transition. Orthodox agricultural methods are totally inadequate. Even with heroic measures like vast jungle clearance and swamp drainage programs or extensive algal cultivation (all of which would take years, hence have increasingly greater deficits to make up), the inevitable is only being postponed.

The same is true of all the ingenious schemes for making economically possible what is now impossible via developments of atomic fission or fusion

power or solar energy. Unless adequate birth control programs are implemented, the population will just keep up with, or stay somewhat ahead of, the food supply and people will live in poverty.

Public opinion surveys in India and Japan show that the majority of the women questioned are in favor of birth control programs. But various outside influences militate against them. The Communists are opposed to such programs. In Western nations the Roman Catholic church is a strong influence against them (other than by a highly inefficient "natural" method). The moral question of whether it is desirable to do good now (e.g., by sanitation, etc., so lowering the death rate) with the prospect being that much evil will be done in the future (by low living standards and starvation) should be considered.

Effective birth control methods are available. Unless far more decisive and effective efforts are made to spread their use, it will be only a short time before there is literally standing room only on earth.

MALCOLM S. GORDON

BOOK REVIEWS

SEGMENTAL ANATOMY OF THE LUNGS. By Edward A. Boyden. New York, McGraw-Hill Book Company, 1955. 276 pp. $15.00.

When the reviewer attempts to recall his experiences in gross anatomy as a medical student some thirty years ago, he recollects little if any time having been spent on the lungs beyond an enumeration of the five lobes and the fissures which define their borders. At this simple level the interests of both instructor and student appear to have been satisfied. However, thirty years is quite a long time. With increasing interest in thoracic surgery after World War I, anatomy of the thorax kept pace with clinical developments, and the study of the lungs themselves now is climaxed by this superb monograph of Edward A. Boyden, Professor Emeritus of Anatomy at the University of Minnesota.

The most effective stimulus to systematic developments in anatomy has always been the needs of the surgeon. This is certainly true of bronchovascular anatomy. Boyden in the introduction to this monograph graciously acknowledges the impetus provided by his surgical colleagues at Minnesota.

The modern era of dissection and resection of pulmonary segments begins in 1939 with Churchill and Belsey's description of lingulectomy. Today no segment is exempt from selective resection when the occasion requires; for example, in the treatment of localized bronchiectatic or tuberculous lesions. After an exhaustive eight-year study of dissection specimens numbering from 50 to 110 for each of the five lobes, Boyden and his colleagues have put the anatomical basis for segmental resections on a firm scientific footing by describing not only the prevailing bronchial and vascular patterns, but the recognized deviations as well.

In the first chapter entitled, "Historical Review," the author does a great service by presenting a comparative analysis of previous attempts to systematize the inner anatomy of the pulmonary lobes. In the second chapter he defines an original and logical system of numerical classification for bronchi (B), pulmonary arteries (A), veins (V) and their divisions through the subsegmental levels. Thus, for example, the pulmonary arterial branch, designated as A^2a2, is recognized by the initiated as a small branch accompanying the inferior subramus (B^2a2) which arises from the posterior ramus (B^2a) of the anterior segmental bronchus (B^2) in the upper lobe.

Each of the five pulmonary lobes is next the subject of an individual chapter in which the surface relationships, bronchial, arterial, and venous patterns are considered in this stated order, with attention to both prevailing and less usual patterns. Finally, a most interesting and illuminating chapter is presented on the embryology of the human lung, utilizing the microdissection method as a tool for the evaluation of congenital anomalies and variations in the adult pattern.

Naturally a work of this inherent complexity and great detail in subject matter does not make easy reading, but the text is carefully edited and lucidly presented. The orderly arrangement of material by chapters and sections makes for ready reference on specific problems. The book is profusely illustrated with pen and ink drawings, color plates, and statistical tables.

The Boyden monograph should be available to every anatomist, thoracic surgeon, and diagnostician. It has occurred to this reviewer that a condensed version of the text, including only those sections concerned with surface anatomy and bronchial patterns, would be of great practical value to otolaryngologists and radiologists who are less frequently concerned with the finer anatomical details of the pulmonary vasculature.

GUSTAF E. LINDSKOG

MECHANISMS OF MICROBIAL PATHOGENICITY. Edited by J. W. Howie and A. J. O'Hea. Cambridge University Press, 1955. x, 333 pp. $5.00.

This volume, containing 16 papers given during the 5th Symposium of the Society for General Microbiology held at the Royal Institution, London, in April 1955, is rewarding reading for all interested in the baffling questions of how micro-organisms cause disease.

Following A. A. Miles' stimulating chapter on "The Meaning of Pathogenicity," sections are devoted to the toxins (with especial attention to those active in diphtheria, gas-gangrene, tetanus, and botulism), and to the tubercle bacillus, *Bacillus anthracis*, and *Pasteurella pestis*. There are chapters on the protoöa and other parasites, on the fungi, and on pathogens of plants. Should the reader question the omission of a discussion of viruses, he is reminded that the second symposium was devoted entirely to these.

The emphasis throughout is directed towards the processes and mechanisms whereby infectious agents produce their effects on the host, and the contributions, each by a worker active in the field concerned, not only are excellent reviews but also contain much new material and fresh speculations.

The organizers of this 5th symposium are to be congratulated for the program, and the editors especially applauded for the speed with which the publication has reached us.

PHILIP B. COWLES

ANALYTICAL CYTOLOGY. By Robert C. Mellors. New York, London, The Blakiston Division, McGraw-Hill Book Company, Incorporated, 1955. 528 pp. $15.00.

Analytical cytology is the acme of a dynamic field which integrates the most recent investigations of chemistry and physics with cellular morphology and physiology. The fields of cytology, histo-chemistry, and cytochemistry are relatively recent in scientific investigation. Therefore, it is appropriate at this time to incorporate in one volume the most recent studies of these "infant sciences," especially by an author who in his own ends avows published articles on the microfluormetric and microspectroscopic methods in cytology and cytopathology.

Dr. Mellors has brought together important contributors who are investigative specialists in their own scientific fields. The book is divided into two main sections: "Methods in the Optical Spectral Region" and "Methods Using Electrons, Radioactive Isotopes and X-rays." The chapters on the

optical spectrum describe cytophotometric analyses, histo-chemical and cyto-chemical staining techniques, contrast, polarizing and fluorescence microscopy. The application of various forms of radiant energy are described in the chapters on electron microscopy, radio-autography, histo-radiography and x-ray diffraction.

Each chapter is well balanced with historical, theoretical, and practical application of each method. I was especially interested in the interpretations and future prospects of each system utilized as presented by the individual authors. Certainly, there are limitations qualitatively and quantitatively applied in each system as discussed by each author. These perplexing problems add incentive to the scientific investigator. The reader's attention is caught by the addenda and references listed of literature which appeared while the book was in publication. The extensive bibliography included after each chapter is excellent. This is a necessity in such a rapidly progressing, dynamic science.

The manner of presentation and contents will be particularly appealing to the research investigator whether at the technician, undergraduate, or graduate level. The large print, mathematical equations, and chemical formulae are easily readable. The charts and curves are meticulous in detail. The diagrams and pictures are good reproductions. The indexing system and numbering of pages and figures is unique and confusing.

I recommend this book to histochemists, anatomists, pathologists, and physiologists. Certainly it should be of interest to physicists, chemists, radiologists, and biological industrial scientists.

ERNEST M. IZUMI

ADAPTIVE HUMAN FERTILITY. By Paul Henshaw. New York, McGraw-Hill Book Co., Inc., 1955. 322 pp. $5.50.

In *Adaptive Human Fertility* Dr. Henshaw has accumulated a diffuse and widely separated potpourri of factual information for the purpose of explaining the reasons for our rapidly increasing human population and a rational approach towards its control. His sources extend all the way from Frazer's *Golden Bough* (although he does not actually quote from this anthropological encyclopedia) to the modern population studies of Pearl, Dublin, and others. The author's apology for the "raison d'etre" of his monograph is capably summarized at the end of one of his chapters when he says "The evidence allows no alternatives. It is necessary to conclude (1) that serious difficulties are arising as a consequence of man's growth in numbers; (2) that effective fertility guidance is essential if social degradation and spreading poverty are to be avoided; and (3) that, to a considerable degree, man's destiny will be determined by his ability to achieve a satisfactory relationship between his reproduction powers and his skill in developing and distributing the materials necessary for life." Dr. Henshaw very ably presents the conflict between man's biological urge to reproduction and the obvious necessity for population control. The inherent conflicts between these two motivations, on the one hand emotional and on the

other intellectual, in our population are discussed, and a possible resolution to the conflict is suggested.

There are some details of this book whose lack of accuracy might disturb the specialized reader, and this reviewer, who is an obstetrician and gynecologist, only hopes that those areas in which he is less informed are more accurate than the sections dealing with aspects of his specialty. It is regrettable, for instance, that the author did not either seek adequate gynecological consultation or submit his manuscript to the clinical perusal of a qualified endocrine gynecologist before publication, because a number of inexact statements in the realm of this specialty could easily have been corrected. We know of no responsible urologist, for example, who has reported fifty per cent success in doing a reanastomosis in the vasectomized male, nor do we know of any satisfactory gynecological experience with the use of the cornual cautery for purposes of sterilization in the female. The fact that it is "as yet not widely used" is not because gynecologists are generally not familiar with it but simply because it has been tried and found wanting. The statement that fifty per cent of cases of infertility are correctable seems to us unduly optimistic, and it would have been of considerable interest in connection with the discussion of various techniques of sterilization to discuss the laws refusing permission for sterilization in many states in this country.

The reviewer, however, does not wish to be captious in his criticism of these and other minor inaccuracies, because the over-all picture presents a point of view which is badly needed in our understanding of present-day population problems. Possibly the author is most capable when he discusses the more theoretical aspects of the problem, especially those concerning desirable population management. Certainly the "welfare optimum" concept is a most useful one, and we feel that the expression "teleo genesis" and the title, *Adaptive Human Fertility*, are most fortunate ones and will rapidly become familiar terms in the specialized language of the demographer.

C. LEE BUXTON

PRINCIPLES OF ANIMAL VIROLOGY. By F. M. Burnet. New York, Academic Press, Inc., 1955. x, 486 pp. $10.00.

In addition to being a text, Dr. Burnet's latest book is a joy to read. Not many scientific writers have the ability to say simply and directly what they mean to say. Dr. Burnet does. Not many scientists have the courage to say what they think without the crutches of their own or others' experimental results. Dr. Burnet does. And in so doing, he dreams up new and exciting studies for himself and others to do. Succinctly put, this is a stimulating account—good to the last page.

The organization of the material is, for the most part, excellent. Perhaps it is premature, however, for viral hemagglutination to be included in a chapter on initiation of cellular infection by viruses; but this might be justified on the grounds that so very little is known about the process(es) of

cellular infection by animal viruses, and thus it is possible that hemagglutination phenomena will lend insight into the manner in which viruses enter cells. Dr. Burnet makes a good case for the influenza viruses in this regard, but he admits that it is difficult to see at present how the two are related in the encephalitis group of viruses, for instance.

There is a very fine chapter on the multiplication of viruses in, and their liberation from, cells; here protein and nucleic acid syntheses are beautifully discussed. More than one statement is provocative of thought, but the following seems especially so: ". . . that living matter has the capacity to produce protein and nucleic acid molecules of such configurations that the only way in which they *can* aggregate is to produce biologically meaningful structures."

Perhaps the best discussion in the book deals with pathogenesis of virus diseases, and within this rubric Dr. Burnet's treatment of neurotropism is especially good. All the pros and cons with regard to neuronal as opposed to hematogenous spread of virus to the central nervous system in natural infections are laid bare and carefully considered. It is here that the author manifests humility .Thus, concerning the pathogenesis of poliomyelitis, he says: "The idea that the apparent spread is due merely to localization of blood-borne virus at a stimulated locus in the central nervous system was not very clearly envisaged by Burnet. . . . in discussing McClosky's . . . findings with poliomyelitis after injection of pertussis vaccine, but has been much more clearly stated by Field. . . ."

In addition to the subjects already mentioned, the work covers the history of animal virology, changes experienced by infected cells, interference, immunology, latent infections, viral neoplasia, ecology of viruses, variation and genetics, virulence, evolution, and finally, classification and nomenclature, the last being a gratifyingly short treatment.

One of the reasons the book is pleasant to read is that the format is easy to look at; the proofreading is occasionally easy-going—"anthropod" instead of "arthropod," "ages" instead of "agents."

At this point it might be well to mention an amusing academic argument that this reviewer has been bold enough to undertake with Dr. Burnet. On page 55 Dr. Burnet says: "The word 'virus' comes from the Latin where it is a noun meaning approximately venom or similar poisonous fluid and being incapable of forming a plural." Cursory examination of the classical material at hand has revealed that, in addition, "virus" was used literally for such vile substances and stenches that quite possibly Latin authors were loathe to employ it except perhaps in a figurative sense. "Venenum" seems to have been a more polite, more poetic, although weaker word used to convey the meaning of poison. It is conceivable that the complete declension of "virus" (viri, virorum, etc.) did indeed exist, but that these were more frequently spoken than written forms, and thus records may show the word to be a defective one, whereas it might be the records that are defective.

This argument is perhaps being carried on for the sole purpose of entertaining the debaters, but that it is being carried on at all attests to the stimulating nature of Dr. Burnet's book.

LISBETH M. KRAFT

SOCIAL SCIENCE IN MEDICINE. By Leo Simmons and Harold G. Wolff. New York, Russell Sage Foundation, 1954. 254 pp. $3.50.

In these times, when our social and political inadequacies in dealing with the advances wrought by nuclear fission are so apparent, it is encouraging to find that forward-looking men in medicine and the social sciences are working together to bridge that gap between their respective fields of interest. Although the authors point out that the practice of medicine at large or in the hospital in particular provides a laboratory in which the social scientist may test his theories, their major thesis is that medicine can no longer afford to overlook the information and aid available from the social sciences.

The biophysical repercussions of famine and war are obvious, and in recent years the psychological factors contributing to gastrointestinal and skin disorders among others have been recognized. Yet the subtler influences of socio-cultural dynamics on biological health have either been overlooked or ignored. It can scarcely be contested that stress, be it physical or psychological, affects the biological functions of man, either in a transitory manner or, if prolonged enough, with permanent damage. The point is that the particular socio-cultural system is a stress inducing environmental factor. To be sure these factors vary according to the socio-cultural background and psychological characteristics of the individual, but they can be investigated, evaluated, and brought into consideration in the treatment of the patient. If these stress-inducing factors are not controlled or if they are inadvertently increased, the patient's disease is not adequately treated, much less the patient as a person. It is in this realm in particular —that of the links between stress and disease— that the authors open up avenues for investigation. Some of the beginning spadework has been done, but the possibilities are almost limitless.

The audience for this volume is not limited to a few because of technical jargon; the text is within the scope of any intelligent and interested person. The extensive bibliography at the end attests to the authors' stated purpose that "the book is addressed primarily to students in medicine and the social sciences interested in training for research in these related areas." It is, furthermore, the reviewer's opinion that students in medicine, by reading this book, may well profit in the practice of their chosen profession simply through the awareness they will gain.

L. Z. B.

PERINATAL MORTALITY IN NEW YORK CITY. Analyzed and reported by S. G. Kohl. Published for the Commonwealth Fund. Cambridge, Massachusetts, Harvard University Press, 1955. xiii, 112 pp. $2.50.

This book is the report of a detailed study conducted by the Subcommittee on Neonatal Mortality, Committee on Public Health Relations, the New York Academy of Medicine, of 955 perinatal deaths in New York City in 1950. A previous publication, *Infant and Maternal Care in New York City*, reported the initial phase of this study and was concerned with hospital

facilities for infant and maternal care. Interest in the causes of perinatal mortality and in their prevention is of great importance since neonatal mortality has not demonstrated a decrease in recent years comparable to that in older age groups. Recent publications by Bundeson and by Potter have also served the commendable purpose of directing increased and much needed attention to the problem of infant deaths.

The present report appraises three categories of infant deaths: stillbirths, neonatal deaths within the first five days, and neonatal deaths from the sixth to the thirtieth day. Each death was reviewed by a team composed of an obstetrician, a pediatrician, and a pathologist for the presence of one or more of the following responsibility factors: inadequate prenatal care, error in medical judgment, error in medical technique, family at fault, unqualified medical attendant, unavoidable disaster, or inadequate pediatric care.

One of the startling conclusions reached in this study was that 35% of these perinatal deaths were preventable! As an example, it was considered that 40% of the infant deaths following delivery by cesarean section were preventable. The report contains a number of other important and disturbing findings all of which have implications for the improvement of perinatal care.

This study would have been even more valuable had a comparable analysis and study been made of infants who had survived. Also, although perhaps not included in its purpose, a discussion of means to diminish the number of preventable deaths would have been of interest. Suggestions for future studies of this character are contained in this report and will be helpful in the planning for similar studies in other communities.

MORRIS GREEN

SHOULD THE PATIENT KNOW THE TRUTH? Samuel Standard and Helmuth Nathan, Eds. New York, Springer Pub. Co., 1955. 159 pp. $3.00.

Physicians, clergymen, nurses, and lawyers contribute chapters to this interesting and valuable little book. Each attempts to present a point of view about a troubling problem: "Should the patient know the truth?" Most of the discussion deals with the care of the patient who has an incurable and fatal disease.

The tenor of most contributions is that no hard and fast rule can be followed; some patients will benefit from a sympathetic but honest appraisal of their prognosis, whereas others would be needlessly hurt by a completely truthful estimate of what lies ahead. Repeatedly it is emphasized that a decision should be made on this point only after careful consideration and full discussion with relatives, nurses, and all others who will have to do with the patient. There are, however, some interesting differences in point of view. A Catholic nurse advocates frankness in all instances, with the comment that: "sparing the patient knowledge of the truth savors of misguided sentimentality. . . ." A Catholic priest, on the other hand, feels that the answer must often depend on circumstances. A divergence also appears in the opinion of two well-known surgeons: Henry Cave and Owen

Wangensteen. Dr. Cave subscribes to the belief that "not to know the truth will cause the least mental anguish to many patients. . . ." Dr. Wangensteen, on the other hand, makes a strong plea for telling the truth, saying that the patient sooner or later guesses what is being hid from him and then loses trust in his physician and is bewildered and hurt by the insincere behavior of his friends and family.

As might be expected when two dozen people are invited to submit chapters, the various contributions in this book are uneven. Dr. Snapper, a physician, responds with a single inconclusive and uninformative paragraph. Dr. Bluestone, a hospital administrator, wanders around for a time and concludes with something on the virtue of obtaining permission for autopsy. The two chapters written from the legal point of view have little value here. Generally, however, the discussions are worth while; and the contributions of Samuel Standard, a physician, and Ilse Wolff, a nurse, are especially good.

PAUL B. BEESON

CULTURAL DIFFERENCE AND MEDICAL CARE. The case of the Spanish-speaking people of the Southwest. By Lyle Saunders. New York, Russell Sage Foundation, 1954. 317 pp. $4.50.

Dr. Saunders, a sociologist from the Department of Preventive Medicine and Public Health at the University of Colorado Medical School, has provided the medical profession with a very stimulating book. The basic premise is that the practice of the healing arts, a social activity, "is determined not only by the knowledge, skills, feelings and attitudes of the professional practitioner" but "also to a considerable extent by the knowledge, feelings and attitudes of the patient, his relatives and friends." Therefore "a knowledge of the cultural and subcultural orientation" of a patient and its possible relation to his behavior in a clinical situation is an important tool in the physician's armamentarium. This is not to imply that we all should become social scientists but rather that an awareness be developed.

The medical and cultural data presented with regard to Spanish speaking people of the American Southwest has the dual rôle of supplying specific information and serving as illustrative material for the discussion of broader social science principles. The first three of the total six chapters are devoted to a social analysis of the historical, cultural, and demographic backgrounds. It is presented in a clear, straight forward style which does not seem to sacrifice accuracy for simplicity. This body of information should be of great interest to those whose work is with these people. The fourth chapter, a most illuminating and fascinating section, is a discussion of the ingredients which make up the knowledge of illness and its treatment which these individuals may have.

The final chapters are a summing up. Two county health programs are evaluated which, though medically sound, failed socially. Requisite and desirable features for future programs are presented. Specific suggestions are made for working with the Spanish-speaking people of the Southwest.

Finally a plea is made for a greater use of the social sciences in the planning of health programs especially where other cultures are involved. "It is not enough to provide services or facilities . . . and hope that they will be taken advantage of" nor to conform to the ideal but rather "the true test of a medical program is . . . whether it works and if so how well."

P. B., JR.

LABORATORY IDENTIFICATION OF PATHOGENIC FUNGI SIMPLIFIED. By Elizabeth L. Hazen and Frank Curtis Reed. Springfield, Ill., Charles C Thomas, 1955. 108 pp. $5.50.

As stated by the authors, the objective of this monograph is to furnish "an aid to the teaching of the essentials in the identification of the pathogenic fungi to the beginner, and a bench companion for the bacteriologist engaged in mycologic diagnosis." This objective is admirably accomplished. The fungi discussed are limited to the pathogenic fungi encountered in North America, being, with few exceptions, members of the class of Fungi Imperfecti. Their means of identification consists of a description of the genus, together with descriptions of the various species. These are correlated with growth characteristics, macroscopic and microscopic, on media selected for growing identifying forms. In addition, there are black and white photographs of the colony formations and photomicrographs of culture forms. Such information is presented for each species discussed. The descriptive portion of the monograph names very briefly the type of disease caused by the species. The descriptions of the cultural and microscopic characteristics are concise and adequate. The appendix contains recipes for the various culture media. The photographs are technically good and the bibliography is extensive.

JOHN C. HALEY

POMP AND PESTILENCE: INFECTIOUS DISEASE, ITS ORIGINS AND CONQUEST. By Ronald Hare. New York, The Philosophical Library, Inc., 1955. 224 pp. $5.75.

Parasites—using the word in its broadest and most general sense—their development of pathogenicity, their effect on humans both singly and collectively, their influence on great moments in history, and modern science's attack on them is here made the subject of a small volume. With this broad topic the author has produced an interesting if thin book for the layman. Many interesting facts are brought forth. But due to the book's brevity they are often strung like beads with little discussion or presentation of evidence. The story ends on a rather gloomy Malthusian note. This reader wishes the author had concentrated his abilities and pleasant style on any one of the many topics covered rather than on this diffuse book.

P. B., JR.

THE DETERMINATION OF ADRENOCORTICAL STEROIDS AND THEIR METABOLITES. (Memoirs of the Society for Endocrinology, No. 2.) Proceedings of a Conference held by the Society, May 21, 1953. Edited by P. Eckstein and S. Zuckerman. London, Dennis Dobson, Ltd., 1953. 91 pp. 12s.6d.; also New York, Cambridge University Press, 1955. $2.75.

This short symposium attempts to collate the methodology current in 1953 for studies of the adrenocortical steroids. As has become the custom at meetings of this nature, little original material is presented. A notable exception to this is the preliminary report by Simpson and Tait of their isolation of electro-cortin, or aldosterone as it is now called, from adrenocortical extracts and of the physico-chemical methods available for its detection. Of interest, too, is the method described by Edwards, Kellie, and Wade for the extraction of steroid conjugates from urine under mild conditions. The excellent discussions by Gray and Lunmon, and by Schwarz, of their experiences with chromatographic methods for examination of steroid mixtures are also to be commended to workers in this field. In view of the price, however, it is doubtful whether this well-printed but brief collection of papers can compete for a place on the bookshelf of the interested investigator with other contemporary reviews of the problem.

MARK A. HAYES

ORIGINS OF RESISTANCE TO TOXIC AGENTS. M. G. Sevag, R. D. Reid, and O. R. Reynolds, Eds. New York, Academic Press Inc., 1955. xv, 471 pp. $12.00.

This symposium, held in March of 1954, was sponsored jointly by the Office of Naval Research and the University of Pennsylvania. The ambitious scope of its coverage can be placed in some perspective by reference to an appendix stating very broad underlying questions and by reference to the concept of the senior editor, stated briefly in the preface and in greater detail in his paper, that "the phenomenon of resistance to various toxic agents can be brought into common focus." Many will fail to see a common basis for resistance to microbicides, herbicides and insecticides, tolerance and addiction, resistance to infection, the mechanism of carcinogenesis and the nature of tumor immunity. However, the comprehensive presentation of these various topics is both stimulating and informative.

The part of the symposium which attempts to deal with the origins of resistance concerns the area in which microbial techniques permit a more detailed analysis. Good evidence is presented which supports, in specific instances, the theory of mutation and selection and also that of adaptation induced by contact with the drug. Yet this part which approaches the heart of the problem is both controversial and confusing. It is not a constructive controversy since there was no agreement on definitions or on the interpretations of basic tests for the occurrence of spontaneous mutations. A. C. R. Dean emphasized his view that the Luria Delbruch interpretation of the fluctuation analysis is open to considerable doubt. The recorded discussion indicated general agreement with respect to the validity of the

Lederberg replica plate test (which yields the same answer as the fluctuation test) as a direct and conclusive demonstration of spontaneous mutation to drug resistance. However, M. G. Sevag and G. T. Lam, in an addendum which was not presented during the symposium (referred to repeatedly throughout the volume), present data which they feel "show clearly that results obtained with the replica plate test do not offer any proof in regard to the spontaneous origin of drug-resistant mutants." It is unfortunate that a problem of semantics detracts from the usefulness of this symposium for students and those concerned with the implications of microbial studies. The proponents of the adaptation theory found an ally in C. P. Martin who presented an address, well documented with interesting biological observations, advancing the theory "that in natural evolution new Mendelian characters arise, usually or always, by means of lingering modifications and not by mutations."

This symposium represents a commendable effort to approach the problem of resistance in its broadest aspect and to achieve a degree of cross-fertilization of ideas from workers in various fields. A number of outstanding investigators have presented detailed reviews of widely separated fields (natural resistance, drug resistance, tolerance and addiction) which make this volume a useful reference. The repeated emphasis on the paucity of direct information concerning the characteristics of resistant cells should stimulate studies of the biochemical and physiological basis of resistance.

CHARLES A. NICHOL

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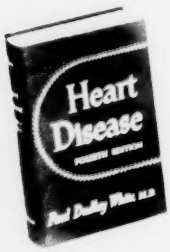

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